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THE PHILIPPINE
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B. TROPICAL MEDICINE

VOL. VIII AUGUST, 1913 No. 4

EXPERIMENTAL ENTAMŒBIC DYSENTERY

By ERNEST LINWOOD WALKER

with the coöperation of

ANDREW WATSON SELLARDS [1]

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

One plate

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PART I. INTRODUCTION

By ERNEST LINWOOD WALKER

Entamœbic dysentery is, with the possible exception of malaria, the most widespread of the endemic tropical diseases, and it has been said to constitute one of the chief obstacles to the white man colonizing in tropical countries. Musgrave wrote in 1904 that amœbic dysentery caused more than 50 per cent of the invalidism of public servants in the Philippine Islands. Fortunately, owing to the great improvement in san-

[1] Joint author on parts II and III.

itary conditions, the percentage of morbidity due to this cause is not so high at the present time, at least in the city of Manila. Gauducheau (1912), at the last biennial congress of the Far Eastern Association of Tropical Medicine, stated that amœbiasis, under the form of dysentery and abscess of the liver, causes nearly half of the deaths of Europeans at Tonkin, Indo China. While it is impossible to give accurate statistics, owing to the fact that the different types of dysentery are not usually separated in the reports of infectious diseases, it is probable that, except where modified by sanitation, this disease holds a more or less similar position in the morbidity and mortality statistics of other tropical countries.

Notwithstanding that amœbæ have been associated with a certain type of dysentery since 1875, the etiologic relation of these organisms to this disease has been the most controverted question in tropical medicine. Between the extreme views that amœbæ combat disease and are the true guardians of man's health (Cassagrandi and Barbagallo, 1895), on the one hand; and, on the other hand, that all amœbæ are or may become pathogenic (Musgrave and Clegg, 1904), every intermediate opinion of the etiologic relation of amœboid organisms to endemic tropical dysentery has been expressed. However, in recent years it is becoming more and more generally accepted that amœbæ do play a rôle in the production of this type of dysentery; yet dissenters from this view still appear from time to time (Tanaka, 1910, and Duncan, 1912), and it has not been definitely proved whether this rôle is primary or secondary.

Our knowledge of the specific amœba concerned in the production of this disease is equally uncertain. Eighteen species representing 4 genera of amœboid organisms [2] have been described as parasites in the intestinal tract of man. Of these at least 5 [3] have been definitely stated to be more or less pathogenic, and there exists no conclusive evidence to exclude the other 11 from the list of pathogenic species. Moreover, the observations and

[2] *Amœba sp.* Noc (1909), *A. limax* (*Vahlkampfia*) Chatton and Lalung-Bonnaire (1912), *Entamœba coli* Schaudinn (1903), *E. histolytica* Schaudinn (1903), *E. undulans* Castellani (1905), *E. tropicalis* Lesage (1905), *E. tetragena* Viereck (1907), *E. phagocytoides* Gauducheau (1908), *E. minuta* Elmassian (1909), *E. nipponica* Koidzumi (1909), *E. tetragena* (non *E. tetragena* Viereck) Akashi (1911), *E. sp.* Akashi (1911), *E. williamsi* Prowazek (1911), *E. brasiliensis* Beaurepaire Aragao (1912), *E. hartmanni* Prowazek (1912), *E. butschlii* Prowazek (1912), *Paramœba hominis* Craig (1906), and *Chalamydophrys stercorea* Cienk. (Schaudinn, 1903).

[3] *Amœba sp.* Noc, *Entamœba histolytica* Schaudinn, *E. tetragena* Viereck, *E. minuta* Elmassian, and *Paramœba hominis* Craig.

experiments of several investigators [4] have led them to believe that amœbæ from water and other nonparasitic sources are capable, when taken into the intestine of man and other animals, of becoming facultative parasites, and in certain cases at least of causing dysenteric symptoms and ulcerative lesions in their host.

In a recent paper (Walker, 1911) I have attempted to establish the morphological distinction between the nonparasitic and the parasitic amœboid organisms and to differentiate the nonpathogenic from the pathogenic species. The conclusions reached in this morphological study include the following which bear directly upon this experimental investigation.

1. The amœboid organisms found in the Manila water supply and other nonparasitic sources belong to the genus *Amœba* Ehrenberg.

2. The amœboid organisms cultivable from the intestinal tract, both of healthy persons and of cases of amœbic dysentery, also belong to the genus *Amœba*.

3. The species of the genus *Amœba* are not parasitic in the intestinal tract of man. When obtained in cultures of stools, they are probably derived from cysts of amœbæ that have been ingested with water or food and have passed unchanged through the intestine.

4. The amœboid organisms parasitic in the intestinal tract of man belong to a distinct genus, *Entamœba* Casagrandi and Barbagallo.

5. The entamœbæ are strict or obligatory parasites, and are incapable of multiplying outside of the body of their host. They cannot be cultivated on Musgrave and Clegg's medium.

6. One nonpathogenic species, *Entamœba coli* Schaudinn, parasitic in the intestinal tract of man, which includes *Entamœba nipponica* Koidzumi,[5] and which develops cysts containing 8 (or more) nuclei, is recognized.

7. One presumably pathogenic species, *Entamœba histolytica* Schaudinn, which includes *"Entamœba tetragena"* Viereck and *"Entamœba minuta"* Elmassian and which develops cysts containing 4 nuclei, is recognized.

[4] Kartulis (1891), Celli and Fiocca (1894), Musgrave and Clegg (1904), Noc (1909), Williams and Gurley (1909), Greig and Wells (1911), Gauducheau (1912), and Chatton and Lalung-Bonnaire (1912).

[5] Hartmann in a recent paper (1912) has concluded that the "nipponica" type of entamœba represents degenerative changes in either *Entamœba coli* or *Entamœba histolytica* (*"E. tetragena"*). A more extended observation leads me to believe that this conclusion is correct.

A number of other authors, notably Schaudinn (1903), Craig (1905), Vedder (1906), Werner (1908), Hartmann (1908), Whitmore (1911), and Darling (1912), have each arrived at some of these conclusions.

The investigation which forms the subject of this paper has been undertaken to test experimentally the validity of these conclusions and to obtain such other information as may be possible concerning the etiology and endemiology of entamœbic dysentery.

Two general methods of experimentation are available for determining the specific identity of microörganisms and their etiologic relation to a disease; namely, (*a*) the study of their immunity reactions and (*b*) the experimental infection of animals.

The application of immunity reactions to the determination of species of amœbæ and of their relations to entamœbic dysentery has been attempted by Sellards (1911). So far as this method was found applicable, it confirmed the conclusions of the morphological study. However, this method of experimentation was found to have certain serious limitations as applied to these organisms. The immunity reactions to protozoa have in general been found to be of a low grade, and those to amœboid organisms present no exception to the general rule. A second and more serious obstacle to the application of these reactions to the problem under consideration is the fact that it has been found impossible to cultivate the parasitic entamœbæ on artificial media or even to keep them alive outside of the body of their host long enough to test the immunity reactions against them. It is further to be noted that immunity reactions can at most only supply indirect evidence of the specific identity of a microörganism and its etiologic relation to a disease.

The experimental infection of animals with amœboid organisms has already been employed very extensively in attempts to prove their etiologic relation to dysentery. Dysentery has been produced by a number of investigators[6] in a certain pro-

[6] Loesch (1875), Hlava (1877), Kartulis (1889), Kovacs (1892), Quincke and Roos (1893), Kreuse and Pasquale (1893), Zancoral (1893), Roos (1894), Gasser (1895), Fijardo (1896), Strong and Musgrave (1900), Harris (1901), Jaeger (1901), Ucke (1902), Huber (1903), Schaudinn (1903), Craig (1905), Hartmann (1908), Werner (1908), Darling (1912), Fantham (1912), Franchini (1912), Hartmann (1912), Wellman (1912), Wenyon (1912).

portion of the experimental animals (cat, dog, monkey) by feeding or injecting rectally dysenteric stools or so-called liver-abscess pus containing entamœbæ. In some cases these experiments have been controlled by feeding to another animal cultures of all of the bacteria that could be grown from the infectious material on ordinary culture media. Dysentery has also been produced experimentally in animals by rectal,[7] and in one case by intravenous,[8] injections of pus from liver abscesses containing entamœbæ, but free from bacteria cultivable on ordinary culture media. Finally, several investigators[9] state that they have produced a disease in animals and, in one case, in man having the clinical symptoms of entamœbic dysentery, with entamœbæ in the stools, and exhibiting the characteristic lesions in the intestine at necropsy, by feeding or injecting rectally "pure mixed cultures" of amœbæ and nonpathogenic bacteria, which had been isolated not only from stools of dysenteric patients, but from water and other nonparasitic sources.

Certain of these experimental infections furnish considerable support to the belief in the etiologic relationship of amœboid organisms to endemic tropical dysentery, but most of the experiments are open to criticism. The infection experiments with dysenteric stools, uncontrolled, are of little more value than clinical observations; since not only the entamœbæ, but all of the other microörganisms contained in the fæces were fed or injected in these experiments. The infections with dysenteric stools, controlled by feeding other animals all of the bacteria cultivable from the stools on ordinary media, and with liver-abscess pus free from bacteria cultivable on ordinary culture media, are more convincing; but they would not exclude bacteria cultivable on special media—or under anaërobic conditions—or the filterable viruses. The work of those authors who obtained dysentery in experimental animals following the feeding of "pure mixed cultures" of amœbæ and nonpathogenic bacteria would appear to obviate all criticism. But these and many of the other experiments are open to a more serious criticism; namely, that the species of amœboid organism fed to, and recovered from, the experimental animal have not been accurately determined. The truth of this statement will be evident to

[7] Kruse and Pasquale (1903), Strong and Musgrave (1900).
[8] Gauducheau (1906).
[9] Kartulis (1891), Musgrave and Clegg (1904), Williams and Gurley (1909).

anyone who takes the trouble to examine the indefinite descriptions of the amœboid organisms employed by these investigators in their experiments. In many instances the author has made no attempt to determine the species with which he has experimented, simply stating that it was an amœba found in a dysenteric stool or cultivated from such a source and that the amœba recovered from the experimental animal resembled, or was indistinguishable from, the amœboid organism fed to the animal. Such experiments supply no information as to the species of amœboid organism associated with the experimental dysentery; it does not prove the etiologic relationship of the associated amœboid organism to the experimental dysentery; and it vitiates every conclusion drawn by the investigator from his experiments.

The use of the lower animals for infection experiments is at best only a makeshift, and the application of the results to man is based on the assumption that the microörganism in question behaves in the experimental animal as it would in the human body, an assumption that is not always borne out by the facts. There may exist a more specific objection to the employment of the lower animals for experiments on entamœbic dysentery. Although a number of authors claim to have been successful in infecting animals and in producing dysentery with different species of amœba and entamœba, I have not been able, in a limited number of experiments, to parasitize animals with the pathogenic entamœba. While these experiments have not been numerous enough to exclude the possibility of infecting animals, they at least indicate that the lower animals are less readily parasitized than man. Every species of animal appears to be parasitized with some species of entamœba, and dysentery is not uncommon in animals kept in captivity. It is probable that these facts, together with carelessness in identifying species of amœboid organisms, account for some of these apparently successful infection experiments on animals with amœbæ and entamœbæ.

The resort to human experimentation is usually not to be recommended, but in certain infectious diseases of wide geographical distribution and prevalence, or which give rise to devastating epidemics, and of which an accurate knowledge of the etiology or transmission cannot otherwise be obtained, experimental infections of man have been resorted to, and the knowledge thus obtained has enabled medical science to control

these diseases to a remarkable extent. The successful transmission of malaria by the anopheles mosquito from man to man (Sambon and Low, 1902) and the experimental infection of man with yellow fever by the stegomyia mosquito (Reed, Carroll, and Agramonte, 1901) are two notable examples of the solution of obscure etiologic and epidemiologic problems through human experimentation and of the vast benefit to mankind that has resulted from such experiments.

Entamœbic dysentery, while it does not cause the spectacular epidemics of some other infectious diseases, is of universal distribution in the Tropics and subtropics, and every year causes a large amount of sickness and death. The study of this disease by clinical and pathological methods and by experiments upon animals has led to no definite agreement upon the etiology or the endemiology of this disease. Therefore, an attempt has been made to determine once for all the specific amœboid organisms, if any, concerned in the production of endemic tropical dysentery by a series of carefully conducted experiments upon volunteers.

These experiments have been carried on within the endemic region at Manila, where material has been available and where the conditions for this investigation have been found to be as favorable as possible. Men at Bilibid Prison, who had long sentences to serve, who had been under observation for years in the prison, and who eat cooked food and drink distilled water exclusively, have been available for the experiments. Moreover, these men had been examined for intestinal parasites, including entamœbæ, on admission to the prison, and those who were infected had received treatment. Consequently, the men have been under complete control, and the existence or possibility of natural infections with amœboid organisms have been reduced to a minimum.

All of the men to whom pathogenic amœboid organisms have been fed were volunteers. The nature of the experiment and the possibility of the development of dysentery as a result of the experiment were carefully explained to each of these men in his native dialect, and each man signed an agreement to the conditions of the experiment written in the native language. No promise of immunity to prison discipline, or commutation of sentence, and no financial inducements were employed to influence a man to volunteer, in accord with the authority under which this work was carried on.

These men were all Filipinos. There appears to exist no definite evidence of a racial immunity of the Filipino to entamœbic dysentery. The disease is endemic in the Philippine Islands, and many Filipinos suffer from it annually. Even if it be granted that a certain degree of immunity does exist, it is believed that the essential results of these experimental infections of Filipinos are applicable to all races of man.

The condition of the men selected for the experiments, with reference to previous attacks of dysentery and to present infections with amœboid organisms, was determined by the clinical history and physical examination of the men and by cultural and microscopic examination of their stools. It was found necessary to include some men who gave a definite history of a mucous and bloody dysentery at some earlier period of their lives. While there is no evidence of the existence of an appreciable immunity following an attack of entamœbic dysentery, the possibility of such a condition was controlled by feeding the same material to other men who had negative dysenteric histories. It is interesting to note in this connection that none of the men who had a positive dysenteric history failed to become parasitized with the pathogenic entamœba. The clinical histories were supplemented in most cases by physical examinations. In some of the later experiments, in which men were used who had negative dysenteric histories, physical examinations were not made. In the majority of the cases, records of one or more examinations of the stools of the men for entamœbæ were available in the hospital records. Cultures on Musgrave and Clegg's medium and microscopic examinations of the stools of all of the men, usually before and after a purgative, were made for amœboid organisms before using them in these feeding experiments. Those men who showed amœbæ after either test, with certain intentional exceptions, were excluded.

In order to cover all of the genera and species of amœboid organisms which were established by the morphological study (Walker, 1911) and which might be concerned in the etiology of entamœbic dysentery, a considerable variety of material has been fed in these experiments. This included all of the species of *Amœba* that could be cultivated from the Manila water supply, from a variety of other nonparasitic sources both within and outside of the Tropics, from the stools of healthy persons, and from the stools of cases of entamœbic dysentery; *Entamœba coli* from healthy persons and of persons suffering from diseases other than

dysentery; cysts of "*Entamœba tetragena*" from "convalescent" and "contract" carriers;[10] and motile *Entamœba histolytica* from acute cases of entamœbic dysentery and from an entamœbic liver abscess.

Each culture of amœba consisted of a single species of amœba, but associated with it was the mixed bacterial growth from the source from which it was cultivated. No attempt was made to isolate the amœbæ in "pure mixed cultures" with one species of bacterium; first, on account of the difficulty of obtaining really "pure mixed cultures"; secondly, since it was impossible to obtain the entamœbæ in "pure mixed cultures," it was considered advisable for the sake of uniformity to feed all of the amœboid organisms with mixed bacterial cultures; and, thirdly, because it was found to be unnecessary.

In the feeding experiments, the growth of amœbæ scraped from the surface of the culture medium and the material containing the entamœbæ, either alone or mixed with powdered starch or magnesium oxide, was inclosed in gelatine capsules and ingested by the men. The powdered starch served to absorb the excess of moisture that would tend to dissolve the gelatine capsule and to facilitate the ingestion of the material. The magnesium oxide served the same purposes as the starch and, in addition, to neutralize the acidity of the contents of the stomach of the experimental man. In every case the capsules of infective material were personally administered.

Following the ingestion of the infectious material, the stools of the men were saved daily until parasitization, or the failure to parasitize, with the specific amœboid organisms was definitely determined, and thereafter at frequent intervals. These stools were examined culturally and microscopically for amœboid organisms, and the species of such organisms, when found, was carefully determined. It has not been considered sufficient to determine the species of amœboid organism fed to, and recovered from, the experimental man, but necessary to follow each case carefully to guard against the possibility of double infection, resulting from a previous latent infection of the man, from impure material used in the feeding experiments or from a sub-

[10] By "convalescent" carrier is meant a person who has suffered from an attack of entamœbic dysentery and has recovered but who is still carrying the specific entamœba; in contrast, the "contact" carrier is a person who, without having had entamœbic dysentery, is carrying the pathogenic entamœba.

sequent natural infection. The clinical symptoms of the parasitized men have been carefully noted, and whenever conditions appeared to warrant a physical examination of them has been made. The men who developed dysentery have been promptly treated after the manifestation of typical clinical symptoms, and all have been cured.

Experimental infections with material such as has been described are subject to certain limitations and sources of error, as are all experiments made with other than pure cultures. Just what these limitations and sources of error are in the present case and how far they can be avoided or controlled is worth considering at this point. In the first place it is to be noted that the presence of other microörganisms in the material fed can in no way interfere with the determination of the parasitism for man of the different species of *Amœba* and *Entamœba*, since they can be identified by their morphological characters in the microscopic examination of the stools. Secondly, it is evident that in feeding experiments with amœboid organisms which are not followed by the development of dysentery, the presence of other microörganisms in the infectious material will not complicate the results. Therefore, it will be possible to eliminate the nonpathogenic species with certainty. Finally, only in infection experiments followed by the development of dysentery will the presence of other organisms in the infectious material be a source of error. In such cases the experiments uncontrolled would not prove the specific amœboid organism to be the primary etiologic agent in the production of the dysentery.

Feeding experiments that were followed by dysenteric symptoms might be controlled by feeding other men all of the bacteria that could be cultivated from the infectious material on ordinary and special media and under aërobic and anaërobic conditions. This would eliminate everything but noncultivable organisms, such as the filterable viruses. However, controls of these experiments were available which made it unnecessary to undertake the work involved in the bacterial cultures and which were considered to be more efficient. It was found that in feeding material containing the presumably pathogenic entamœbæ not all of the men become parasitized with the entamœbæ. Such individuals were equivalent to controls that had been fed not only all of the cultivable but also any noncultivable microörganisms that the infectious material might contain, and they have been reserved as controls of the men fed at the same time with

the same material, but who did become parasitized with the entamœbæ. Furthermore, it has been found that not all of the individuals parasitized with the presumably pathogenic entamœbæ developed dysentery; that is, some of them become "contact carriers." A number of feeding experiments have been made with entamœbæ from such "carriers" who had not, and have not subsequently, developed dysentery. In several cases the entamœbæ have been passed successively through two such "carriers" to a third man, in some of whom dysentery was produced. By these controls the attempt has been made to eliminate the possible etiologic action of the bacteria or other microörganisms associated with the pathogenic entamœbæ.

This large series of experimental infections has been conducted to a successful finish with a minimum of discomfort and without danger to the men. By these experiments it is believed that the specific entamœba concerned in the etiology of endemic tropical dysentery has been definitely determined, the endemiology of this disease elucidated, and information obtained of the greatest value for the diagnosis, treatment, and prophylaxis of this important tropical disease.

PART II. FEEDING EXPERIMENTS WITH CULTURES OF AMŒBÆ

By Ernest Linwood Walker and Andrew Watson Sellards

This series of experiments was undertaken to obtain cumulative evidence refuting the conclusions of several authors that amœbæ cultivated from water and other nonparasitic sources and from dysenteric stools are capable of living parasitically and, in certain cases, of producing dysenteric symptoms and ulcerative lesions in the intestine of man and other animals.

Kartulis (1891) reports the production of dysentery in 1 cat by rectal injections of pure cultures of amœbæ, isolated from a liver abscess, and in 2 cats with impure cultures of amœbæ, isolated from a dysenteric stool, grown on a straw-infusion medium. These experiments were controlled by feeding and injection experiments with the bacteria isolated from dysenteric stools, which were followed by negative results.

Celli and Fiocca (1894) cultivated 6 species of amœbæ from the intestine of man, which they identified with species which they had cultivated from water and soil. No experiments were made to test the pathogenicity of the species.

Musgrave and Clegg (1904) state that they produced dysentery in monkeys, and in one case in man, having the symptoms and lesions of entamœbic dysentery, with amœbæ in the stools, by feeding, or injecting subcutaneously, "pure mixed cultures" of amœbæ and harmless bacteria, which had been cultivated not only from dysenteric stools but also from

washings from lettuce and from the Manila water supply. These authors maintained that all amœbæ are, or may become, pathogenic.

One of us (Walker, 1908) obtained in culture on Musgrave and Clegg's medium an amœba, "*Amœba hominis*," from the intestinal tract of a woman at necropsy which was at that time believed to be a parasitic species. No tests were made of the pathogenicity of this amœba.

Noc (1909) considers the amœbæ found in dysenteric stools, in the sections of dysenteric intestines, and in liver abscesses to be identical with an amœba common in the drinking water in Indo China and which he has cultivated on artificial media. Animal experiments with cultures from both the water and the dysenteric stools gave negative results.

Williams and Gurley (1910) produced attacks of typical bloody dysentery in a kitten by feeding *Amœba limax* cultivated from potato parings, while a kitten fed the bacteria associated with the amœbæ in culture showed no symptoms.

Greig and Wells (1911) believe that the amœba found in the stools of dysenteric patients and in the pus from liver abscesses in Bombay is not *Entamœba histolytica* Schaudinn, but the same species that is found in Cochin China. This same amœba, these authors state, is found in the conduit water of Bombay.

Gauducheau (1912) is of the opinion that the so-called *limax* amœbæ are in size and structure like *Amœba phagocytoides*, cultivated by him in 1907, and found in the intestine of dysenteric cases and in water. These amœbæ, he says, are capable of multiplying in the intestine of animals, and there can be no doubt of their parasitic nature.

Chatton and Lalung-Bonnaire (1913) describe an amœba (*Amœba limax*) which they cultivated from a case of chronic intermittent diarrhœa and which they believe to be the same as the amœboid organism which they found microscopically in the stools of the patient.

One of us (Walker, 1911) has already determined that morphologically the cultivable amœbæ belong to the genus *Amœba* Ehrenberg. Species of this genus are characterized morphologically by the more or less central position of the nucleus in the resting organism, by the arrangement of the greater part of the chromatin of the nucleus in a central karyosome, by the presence (with rare exceptions) of a contractile vacuole, by the development of mononuclear cysts; and, biologically, by the absence of schizogony in the encysted stage, by their ability to live non-parasitically, and to multiply on artificial culture media (Plate I, figs. 1 and 2).

Twenty feeding experiments have been made with cultures from 11 different sources, representing 13 strains and 8 species of *Amœba*, as detailed in Table I.

TABLE I.—*Strains of amœbæ used in feeding experiments.*

Strain No.	Source.	Locality.	Species.	Feedings.
1	Water supply	Manila, P. I.	*A*	2
2	do	do	*A*	8
3	do	do	*B*	1
4	Clover	U. S. A.	*C*	1
5	Hay	Illinois, U. S. A.	*D*	2
6	Algæ	Kansas, U. S. A.	*E*	1
7	Normal stool	Manila, P. I.	*F*	1
8	do	do	*A*	1
9	do	do	*F*	1
10	Diarrhœal stool	Kansas, U. S. A.	*G*	3
11	Dysenteric stool	Manila, P. I.	*G*	1
12	do	do	*H*	2
13	do	do	*F*	1
	Total		8	20

To these 8 distinct species of *Amœba* no names have been given, since to do so in the present chaotic state of the nomenclature of the free-living amœbæ would only add to the confusion. Species *A*, *B*, *F*, *G*, and *H* are illustrated in the plates of an earlier paper by one of us (Walker, 1911). Among these 13 strains of amœbæ are represented all of the different species that could be cultivated from the Manila water supply, from normal stools, and from dysenteric stools in Manila. A larger series of experiments with cultures from dysenteric stools was not considered necessary, in view of the behavior of all of the amœbæ in the intestinal tract of man.

The amœbæ fed in these experiments were cultivated on a medium which had the following composition:

Agar-agar	2.5 grams
Sodium chloride	0.05 gram
Liebig's beef extract	0.05 gram
Normal sodium hydroxide	2 cc.
Distilled water	100 cc.

Without clarifying, it was sterilized at 7 kilograms' pressure per square centimeter for about three-quarters of an hour. After the sterilization, its reaction was neutral to phenolphthalein. This medium, which is essentially that of Musgrave and Clegg (1904), has proved satisfactory, all of the amœbæ growing well upon it.

The cultures of amœbæ used in the feeding experiments were pure with reference to the protozoön; that is, they consisted of a single species of *Amœba*, but they were cultivated with the

mixed growth of bacteria with which they had been isolated. The presence of bacteria growing with the amœbæ in mixed cultures would be objectionable only if the experiments were followed by the development of dysentery, and since all of the cultures of amœbæ were confirmed to be neither pathogenic nor parasitic this objection has no foundation.

The 20 experiments were made on 10 different men, some of them being used for several successive experiments. When the ingested organism failed to parasitize the man, he was used, after the lapse of a sufficient interval and after repeated negative cultural and microscopic examinations of his stools, to repeat the experiment with the same or a different amœboid organism. Since the species of amœba ingested could in every case be identified microscopically, the use of the man subsequently for feeding another species of amœboid organism in no way interfered with the continued observation of him with reference to the former experiment. Such men were in certain respects more desirable for subsequent experiments than new men, for they had been under more immediate observation and the possibility of previous amœbic infection had been more certainly excluded by the large number of cultural and microscopic examinations that had been made of their stools.

Some of the men used in these experiments gave a history of one or more attacks of dysentery at some earlier periods of their lives. Their present condition with reference to dysentery or amœbic infection was determined by physical examination and by cultural and microscopic examination of their stools. The microscopic examinations were made both before and after the administration of a saline purgative. With one exception, men showing any evidence of amœbic infection by either method were excluded from the experiments. One man was already infected with *Entamœba coli* when employed for a feeding experiment with a culture of amœbæ. Use was made of him to control our differentiation of the amœbæ from the entamœbæ by cultural and microscopic examinations.

The amœbæ were fed for the most part in the encysted condition, since this is the resistant stage and seemed most likely to be capable of infecting the men. In a few cases they were fed in the amœboid stage. Some precautions were necessary in the latter case to eliminate the presence of the encysted amœbæ. This was accomplished by making several successive transplants of the cultures to fresh medium at from fifteen to eighteen hours' interval; for it is only in old cultures that the amœbæ become encysted. In feeding experiments with encysted amœbæ, a trans-

plant to fresh culture medium was made in every case to test the viability of the cysts.

The growth scraped from several agar-slant cultures or Petri-plate cultures, sometimes alone, but more often mixed with magnesium oxide to absorb the excess of moisture that would dissolve the gelatine capsule and to neutralize the acidity of the contents of the stomach that might prevent infection, was inclosed in a gelatine capsule and ingested by a man. The motile amœbæ were ingested as an emulsion in water with magnesium oxide. It might be argued that the action of the gastric juices was necessary to dissolve the cysts of the amœbæ, and, consequently, the use of magnesium oxide to neutralize the gastric acidity in feeding the encysted amœbæ would tend to prevent infection. This argument would not be applicable, however, to the cultivable amœbæ. The cysts of these amœbæ are dissolved or ruptured from within whenever they are placed on fresh culture medium or in any medium suitable for growth, and, moreover, acids are extremely antagonistic to the growth of these amœbæ. For these reasons there is no objection to the neutralization of the acidity of the stomach contents of the men used in these experiments.

Following the ingestion of cultures of amœbæ, the stools of the men were examined daily, both culturally and microscopically, for amœboid organisms until the parasitization or non-parasitization with the specific amœba was determined, and thereafter at frequent intervals.

A complete protocol is given of each man in order to put on record the details of these experiments.

Experiment I.—Man 3, aged 31 years, had been under observation in the prison for six years. He gave a history of one attack of dysentery of one month's duration sixteen years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of *Amœba IA*, mixed with magnesium oxide. This amœba was one of the 2 species isolated in culture from the Manila water supply. The culture fed was an old one containing only encysted amœbæ. Transplant cultures made on fresh culture media to test the viability of the cysts gave a luxuriant growth of *Amœba A*. This man has been under observation two years and seven months since the experiment began. Cultures of his stools on Musgrave and Clegg's medium and microscopic examinations of his stools for amœboid organisms have been constantly negative. No dysenteric symptoms have developed.

Experiment II.—Man 4, aged 34 years, had been under observation in the prison one year and six months. He had a negative dysenteric history,

and had not been used previously for feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of *Amœba 2A*, isolated in culture from the Manila water supply, mixed with magnesium oxide. These cultures contained both motile and encysted amœbæ. Transplant cultures made to test the viability of the organisms showed a luxuriant growth of *Amœba A*. This man was under observation eleven and one-fourth months after the experiment began. Following the feeding, cultures of this man's stools on Musgrave and Clegg's medium and microscopic examination of his stools for amœboid organisms were constantly negative. No symptoms of dysentery have developed.

Experiment III.—Man 3, aged 31 years, had been under observation in the prison for six years. He gave a history of one attack of dysentery of one month's duration sixteen years ago. He had been previously used for another feeding experiment with negative results (see experiment I). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœbæ were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba IA*, isolated from the Manila water supply, mixed with magnesium oxide. The cultures ingested by this man were old ones containing only encysted amœbæ. Transplant cultures to test the viability of the cysts gave a luxuriant growth of *Amœba A*. This man has been under observation two years and six months since the experiment began. Cultures of his stools on Musgrave and Clegg's medium and repeated microscopic examinations of his stools for amœboid organisms have been negative. No symptoms of dysentery have developed.

Experiment IV.—Man 4, aged 34 years, had been under observation in the prison one year and six months. He had a negative dysenteric history. He had been used previously for another feeding experiment with negative results (see experiment II). Physical examination of the abdomen and cultural and microscopical examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba 2A*, isolated from the Manila water supply, mixed with magnesium oxide. The cultures ingested contained both motile and encysted amœbæ. This man has been under observation eleven months and seven days since the experiment began, during which time *Amœba A* has never been found in his stools nor have dysenteric symptoms developed.

Experiment V.—Man 2, aged 40 years, had been under observation in the prison five years and one month. He had a negative dysenteric history. He had been used for another experiment sixty-nine days previously with negative results (see experiment VII). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba 2A*, isolated from the Manila water supply, mixed with magnesium oxide. The cultures fed to this man contained encysted amœbæ only. Transplant cultures to test the viability of the cysts showed an abundant growth of *Amœba A*. This man has been under observation two years and four and one-half months since this experiment began. Cultures and microscopic examinations of his stools for amœboid organisms have been constantly negative, and no symptoms of dysentery have developed.

Experiment VI.—Man 7, aged 30 years, had been under observation in the prison for five years and nine months. He gave a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had been used for 3 previous feeding experiments (experiments XI, XIII, and XVI), the last of which was ninety-two days previously, and all of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 7 Petri-plate cultures of *Amœba 3B*, mixed with magnesium oxide. *Amœba B* was the second of 2 species which had been isolated in culture from the Manila water supply. The cultures ingested by this man consisted exclusively of encysted amœbæ. Transplant cultures made to test the viability of the cysts showed an abundant growth of amœbæ. *Amœba B* was recovered in cultures from the stools of this man from the first to the fifth day after ingestion but never subsequently. Microscopic examination of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and one and one-half months since this experiment began. No symptoms of dysentery have developed.

Experiment VII.—Man 2, aged 40 years, had been under observation in the prison four years and ten months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 1 agar-slant culture of *Amœba 4C*, mixed with magnesium oxide. *Amœba C* was isolated from clover grown in the United States. The culture ingested was an old one containing only encysted amœbæ. A transplant culture made from this culture to test the viability of the cysts gave an abundant growth of *Amœba C*. Following the feeding, cultures and microscopic examinations of his stools for amœboid organisms were constantly negative, and no dysenteric symptoms developed. He was under observation one hundred twenty-six days when he was used for a feeding experiment with *Entamœba histolytica* (part IV) and developed dysentery on the twentieth day with *Entamœba histolytica* in his stools. Altogether, this man has been under observation two years and seven months, during which time *Amœba C* has never been found culturally or microscopically in his stools.

Experiment VIII.—Man 8, aged 57 years, had been under observation in the prison for seven years and eight months. He had a history of 1 attack of dysentery of one month's duration eight years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organism were negative. He ingested the growth on 2 Petri-plate cultures of *Amœba 5D*, mixed with magnesium oxide. *Amœba D* had been isolated in culture from an infusion of hay coming from Illinois, United States. The cultures fed to this man contained encysted amœbæ only. Transplant cultures made to test the viability of the cysts showed an abundant growth of *Amœba D*. Following the ingestion, cultures of the stools of this man showed a growth of *Amœba D* on the first day after feeding, but never subsequently. Microscopic examinations of his stools have been constantly negative. This man has been under observation two years and five and one-half months since this experiment began, but has never shown any symptoms of dysentery.

Experiment IX.—Man 3, aged 31 years, had been under observation in the prison for six years and two months. He gave a history of dysentery of one month's duration sixteen years ago. He had been used for 3 previous feeding experiments (experiments I, III, and XV) the last of which was thirty-four days previously, and all of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 agar-slant cultures of *Amœba 5D*, isolated in culture from hay, mixed with magnesium oxide. The cultures ingested by this man contained motile forms of the amœba exclusively. *Amœba D* was recovered in cultures from the stools of this man on the second and third days after feeding, but never subsequently. Microscopic examinations of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and four and one-half months since the experiment began. No symptoms of dysentery have developed.

Experiment X.—Man 9, aged 27 years, has been under observation in the prison for five years and three months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen was negative. Microscopic examination of his stools showed a few *Entamœba coli*. Cultural examinations of his stools for amœbæ were negative. He ingested the growth on 2 agar-slant cultures of *Amœba 6E*, mixed with magnesium oxide. *Amœba E* had been isolated in culture from fresh-water algæ obtained from Kansas, United States. The cultures fed to this man contained encysted amœbæ only. Transplant cultures made to test the viability of the cysts showed a good growth of *Amœba E*. Following the feeding, cultures of this man's stools showed *Amœba E* on the first and second days after feeding, but never subsequently. Microscopic examinations of his stools have constantly showed a few *Entamœba coli*. This man was under observation three months. No symptoms of dysentery developed.

Experiment X demonstrates the morphological and biological differences between the cultivable amœbæ and the parasitic entamœbæ. *Amœba E* was recovered in culture, but could not be found microscopically in the stools of this man; on the other hand, *Entamœba coli* was identified microscopically, both before and after feeding *Amœba E*, but could not be cultivated.

Experiment XI.—Man 7, aged 30 years, had been under observation in the prison for four years and eight months. He gave a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopical and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba 3A*, mixed with magnesium oxide. This strain of *Amœba A*, which is the same species as the amœba common in the Manila water supply, had been isolated in culture from a stool of a healthy man. The cultures fed in this experiment contained only encysted amœbæ. Transplant cultures to test the viability of these cysts showed a luxuriant growth of *Amœba A*. Following the feeding, cultures and microscopic examinations of the stools of this man for amœboid organisms have been

constantly negative. This man was under observation for forty-three days after the experiment began, when he was used for another feeding experiment (experiment XVI). Altogether, he has been under observation for two years and six months. During this time *Amœba A* has never been found microscopically or culturally in his stools, nor has he ever shown any symptoms of dysentery.

Experiment XII.—Man 4, aged 34 years, had been under observation in the prison one year and seven months. He had a negative dysenteric history. He had been used for 2 experiments forty-one and twenty-nine days previously, respectively, with negative results (experiments II and IV). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of *Amœba 7F*, mixed with magnesium oxide. *Amœba 7F* had been cultivated from a stool of a healthy man. The cultures fed in this experiment contained only encysted amœbæ. Transplant cultures to test the viability of the cysts gave an abundant growth of *Amœba F*. Following the ingestion, cultures of this man's stools showed a growth of *Amœba F* on the second day only after feeding. Microscopic examination of his stools for amœboid organisms were constantly negative. This man was under observation ten months after the experiment began. No symptoms of dysentery developed.

Experiment XIII.—Man 7, aged 30 years, had been under observation in the prison for four years and five months. He had a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had been used for a previous feeding experiment with negative results (experiment XI). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of *Amœba 9F* cultivated from the stool of a healthy man, mixed with magnesium oxide. The cultures ingested by this man contained encysted amœbæ only. Transplant cultures made to test the viability of the cysts showed an abundant growth of *Amœba F*. Following the feeding, cultures of this man's stools on Musgrave and Clegg's medium showed a growth of *Amœba F* on the first day, but never subsequently. Microscopic examinations of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and six and one-half months since the experiment began, but has never shown any dysenteric symptoms.

Experiment XIV.—Man 1, aged 29 years, had been under observation in the prison three years and ten months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 3 agar-slant cultures of *Amœba 10G*, mixed with magnesium oxide. *Amœba G* had been cultivated from a diarrhœal stool in Kansas, United States. The cultures ingested by this man were old, and contained encysted forms exclusively. Transplants from each of the cultures fed, made on fresh culture media to test the viability of the cysts, showed an abundant growth of *Amœba G*. Following the feeding, *Amœba G* was recovered in cultures of this man's stools on Musgrave and Clegg's medium on the first and second days after feeding, but never subsequently. Microscopic examinations of this man's stools were made daily, Sundays excepted, for thirty-five days with negative results. On the thirty-fifth day his stool

was still formed, but was surrounded by considerable mucus streaked with blood. Microscopic examination and cultures were negative for amœboid organisms. On the thirty-sixth day his stool was partly formed and partly fluid, the fluid portion consisting of mucus and blood. Three cultures of the material were negative. Microscopic examination showed not *Amœba G*, which had been ingested by the man, but a distinct genus and species of amœboid organism, *Entamœba histolytica* (part IV). The dysenteric condition persisted for only two days, and he recovered without treatment. *Entamœba histolytica* has persisted in this man's stools up to the present time, two hundred forty-one days after the beginning of the experiment. During the period of observation no relapse of the dysenteric symptoms has occurred. At no time has *Amœba G* been found microscopically in this man's stools, nor was it ever recovered in cultures after the first two days subsequent to feeding. This man either had a previous latent infection with *Entamœba histolytica*, or became infected with the pathogenic entamœba subsequent to ingesting *Amœba G*.

Experiment XIV shows several things besides that which it was planned to demonstrate. First, it shows the possibility of latent or secondary infections with other amœboid organisms in such experiments; secondly, it emphasizes the care necessary to exclude such secondary infections in experimental work; thirdly, it illustrates the chief source of error in the conclusions of previous experimenters; and, fourthly, it has demonstrated our ability to exclude such sources of error from our experiments.

Experiment XV.—Man 3, aged 31 years, had been under observation in the prison for six years and one month. He gave a history of dysentery of one month's duration sixteen years ago. He had been used for 2 feeding experiments with cultures of amœbæ, thirty-six and twenty-four days previously, respectively, with negative results (experiments I and III). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba 10G*, isolated from a diarrhœal stool in Kansas, mixed with magnesium oxide. The cultures ingested by this man contained only encysted amœbæ. Transplant cultures made to test the viability of the cysts showed an abundant growth of *Amœba G*. Following the feeding, cultures of this man's stools showed a growth of *Amœba G* on the second day after feeding, but never subsequently. Microscopic examinations of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and five and one-half months since the experiment began. During this time *Amœba G* has never been found microscopically in his stools, and he has never shown any symptoms of dysentery.

Experiment XVI.—Man 7, aged 30 years, had been under observation in the prison for four years and six months. He gave a history of 4 attacks of dysentery, each of one week's duration, six years ago. He had been used for 2 previous feeding experiments (experiments XI and XIII), the latter of which was thirty-four days previously, and both of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for

amœboid organisms were negative. He ingested the growth on 4 agar-slant cultures of *Amœba 10G*, isolated from a dysenteric stool in Kansas, mixed with magnesium oxide. The cultures ingested by this man contained motile forms of the amœba exclusively. *Amœba G* was recovered in cultures of this man's stools on the sixth day only after feeding. Microscopic examinations of his stools for amœboid organisms have been constantly negative. He has been under observation two years and four and one-half months since this experiment began. No symptoms of dysentery have developed.

Experiment XVII.—Man 5, aged 30 years, had been under observation in the prison for six years and nine months. He gave a history of mucous dysentery seven years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 3 Petri-plate cultures of *Amœba 11G*, mixed with magnesium oxide. This amœba was isolated in culture from a man suffering from an acute attack of entamœbic dysentery. The culture fed contained only encysted forms. Transplant cultures made to test the viability of the cysts all showed a growth of *Amœba G*. Cultures of this man's stools showed a growth of *Amœba G* on the first, second, and third days after feeding, but never subsequently. Microscopic examinations of his stools have been constantly negative. This man has been under observation two years and six and one-half months since this experiment began. During this time amœboid organisms were never found microscopically or culturally in his stools, and he has never exhibited any symptoms of dysentery.

Experiment XVIII.—Man 6, aged 27 years, had been under observation in the prison for five years and six months. He gave a history of bloody mucous stools for four months, two years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amœba 12H*, unmixed with other substance. This amœba was isolated in culture from a dysenteric stool. The cultures ingested contained both motile and encysted forms of the amœba. Transplant cultures made to test their viability showed a luxuriant growth of *Amœba H*. Cultures of this man's stools on Musgrave and Clegg's medium showed a growth of *Amœba H* on the first day after feeding, but never subsequently. Microscopic examinations of his stools have been constantly negative. This man was under observation one year and five months following the beginning of this experiment. During this time *Amœba H* has never been found microscopically in his stools, and he has never shown any symptoms of dysentery.

Experiment XIX.—Man 6, aged 27 years, had been under observation in the prison for five years and seven months. He had a history of a bloody mucous dysentery for four months, two years ago. He had been used sixteen days previously for another feeding experiment with the same strain and species of amœba, with negative result (experiment XVIII). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. This man ingested the growth on 4 Petri-plate cultures of *Amœba 12H*, cultivated from a dysenteric stool, mixed with magnesium oxide. The cultures ingested by this man contained encysted amœbæ only. Transplant

cultures made to test the viability of the cysts showed a good growth of *Amœba H*. Cultures of this man's stools showed a growth of *Amœba H* on the first and second days after feeding, but never subsequently. Microscopic examinations of his stools were constantly negative. This man was under observation one year four months and eighteen days after this experiment began. No symptoms of dysentery developed.

Experiment XX.—Man 10, aged 45 years, had been under observation in the prison four years and eleven months. He gave a history of dysentery three years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen disclosed thickened bands along the sigmoid. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 3 Petriplate cultures of *Amœba 13F*, mixed with magnesium oxide. This strain of *Amœba F* had been isolated in culture from a stool of an acute case of entamœbic dysentery in Manila. The cultures of this amœba ingested by this man contained encysted forms only. Transplant cultures made to test the viability of the cysts showed a luxuriant growth of the amœba. *Amœba F* was recovered in cultures from this man's stool from the first to the third day after feeding, but never subsequently. Microscopic examinations of his stools for amœboid organisms were constantly negative. This man was under observation five months after this experiment began. No symptoms of dysentery developed.

The protocols are summarized in Table I.

From the protocols and Table I it will be seen that, with the exception of species *A* and *C*, the specific amœba ingested in these experiments was in every case recovered in cultures on Musgrave and Clegg's medium from the stools of the man to whom they were fed on the first to the sixth day after ingestion, but never subsequently. Species *C* was ingested only once, and was not recovered in cultures. Three strains of species *A*, fed five times, were never recovered in cultures of the stools of the experimental men.

On the other hand, microscopic examination of the stools of these men were, with one apparent exception, always negative, although in many of these experiments the men have been under observation for over two years. The one exception to this result was in experiment IX, in which the man was already parasitized with *Entamœba coli* before ingesting a culture of *Amœba E*. *Amœba E* was recovered in cultures on the first and second days after feeding and not subsequently, but it was never found microscopically in the stools of this man. *Entamœba coli*, on the other hand, was never obtained in cultures, but it was constantly found microscopically in the stools of this man. Thus *Amœba E* behaved like the other amœbæ in the intestinal tract of man, and it was distinguished morphologically and biologically from *Entamœba coli* in this experiment.

TABLE I.—*Feeding experiments with cultures of Amœbæ.*

Experiment No.	Records of men previous to experiment.							Source and description of material ingested.					Time under observation after feeding.	Results of feeding experiments.		
	No.	Age.	Time under observation in prison.	History with reference to dysentery.	Physical examination of abdomen.	Used for previous feeding experiments.	Microscopic examination of stools.	Culture of stools on Musgrave and Clegg's medium.	Source of culture.	Amœba. Strain.	Species.	Stage of development.	Quantity of material fed.	Material fed with—		Culture of stools on Musgrave and Clegg's medium.	Microscopic examination of stools.	Dysentery.
		Yrs.	*Yrs. mos.*												*Yrs. mos. days.*			
I	3	31	6 0	Dysentery 16 years ago	Negative	None	Negative	Negative	Manila water supply	1	*A*	Encysted	Growth on 2 Petri-plate cultures	Nothing	2 7 0	Negative	Negative	Negative.
II	4	34	1 6	Negative	do	do	do	do	do	2	*A*	Motile and encysted	do	do	0 11 7	do	do	Do.
III	3	31	6 0	Dysentery 16 years ago	do	Culture of *Amœba 1A* 12 days previously with negative results.	do	do	do	1	*A*	Encysted	Growth on 4 Petri-plate cultures	Magnesium oxide	2 6 0	do	do	Do.
IV	4	34	1 6	Negative	do	Culture of *Amœba 2A* 12 days previously with negative results.	do	do	do	2	*A*	Encysted and motile	do	do	0 11 7	do	do	Do.
V	2	40	5 1	do	do	Culture of *Amœba 4C* 80 days previously with negative results.	do	do	do	2	*A*	Encysted	Growth on 4 agar-slant cultures	do	2 4 15	do	do	Do.
VI	7	39	5 9	3 attacks of dysentery 6 years ago	do	Culture of *Amœba 8A* 126 days previously; culture of *Amœba 9F* 127 days previously; culture of *Amœba 10G* 93 days previously. Results of all three feedings negative.	do	do	do	3	*B*	do	Growth on 7 Petri-plate cultures	do	2 1 15	*Amœba B* recovered on first and fifth days after feeding	do	Do.
VII	2	40	4 10	Negative	do	None	do	do	Clover, U. S. A	4	*C*	do	Growth on 1 agar-slant culture	do	2 7 0	Negative	do	Do.
VIII	8	37	7 9	1 attack of dysentery 3 years ago	do	do	do	do	Hay, Illinois, U. S. A	5	*D*	do	Growth on 2 agar-slant cultures	do	2 5 15	*Amœba D* recovered on first day after feeding	do	Do.
IX	3	31	6 2	Dysentery 16 years ago	do	Culture of *Amœba 1A* 70 days previously; culture of *Amœba 10C* 34 days previously. Results of both feedings negative.	do	do	do	5	*D*	Motile	Growth on 4 agar-slant cultures	do	2 4 15	*Amœba D* recovered on second and third days after feeding.	do	Do.
X	9	28	4 3	Negative	do	None	Motile *Entamœba coli*	do	Algæ, Kansas, U. S. A	6	*E*	Encysted	Growth on 2 agar-slant cultures	do	0 3 0	*Amœba E* recovered on first and second days after feeding	Encysted *Entamœba coli*[a]	Do.
XI	7	30	4 3	3 attacks of dysentery 6 years ago	do	do	Negative	do	Normal stool, Manila	8	*A*	do	Growth on 4 Petri-plate cultures	do	2 6 2	Negative	Negative	Do.
XII	4	34	1 7	Negative	do	Culture of *Amœba 2A* 36 days previously with negative results; culture of *Amœba 2A* 24 days previously with negative results.	do	do	do	7	*F*	do	Growth on 2 Petri-plate cultures	do	0 10 2	*Amœba F* recovered on second day after feeding	do	Do.
XIII	7	30	4 5	3 attacks of dysentery 6 years ago	do	Culture of *Amœba 8A* 9 days previously with negative results.	do	do	do	9	*F*	do	do	do	2 5 15	*Amœba F* recovered on first day after feeding	do	Do.
XIV	1	29	3 10	Negative	do	None	do	do	Diarrhœal stool, Kansas, U. S. A	10	*G*	do	Growth on 3 agar-slant cultures	do	0 8 0	*Amœba G* recovered on first and second days after feeding	do	Do.[b]
XV	3	31	6 1	Dysentery 16 years ago	do	Culture of *Amœba 1A* 36 days previously; culture of *Amœba 1A* 24 days previously. Results of both feedings negative.	do	do	do	10	*G*	do	Growth on 4 agar-slant cultures	do	2 5 15	*Amœba G* recovered on second day after feeding	do	Do.
XVI	7	30	4 6	3 attacks of dysentery 6 years ago	do	Culture of *Amœba 8A* 43 days previously; culture of *Amœba 9F* 34 days previously. Results of both feedings negative.	do	do	do	10	*G*	Motile	do	do	2 4 15	*Amœba G* recovered on sixth day after feeding	do	Do.
XVII	5	30	3 9	Mucous dysentery 7 years ago	do	None	do	do	Dysenteric stool, Manila	11	*G*	Encysted	Growth on 3 Petri-plate cultures	do	2 6 15	*Amœba G* recovered on first to third days after feeding	do	Do.
XVIII	6	27	5 6	Bloody mucous stools 2 years ago	do	do	do	do	do	12	*H*	Motile and encysted	Growth on 4 Petri-plate cultures	Nothing	1 5 0	*Amœba H* recovered on first day after feeding	do	Do.
XIX	6	27	5 7	do	do	Culture of *Amœba 12H* 16 days previously with negative results.	do	do	do	12	*H*	Encysted	Growth on 4 agar-slant cultures	Magnesium oxide	1 4 18	*Amœba H* recovered on first and second days after feeding	do	Do.
XX	10	46	4 11	Dysentery 3 years ago	Thickened bands along sigmoid.	None	do	do	do	13	*F*	do	Growth on 3 Petri-plate cultures	do	0 5 23	*Amœba F* recovered on first to third days after feeding	do	Do.

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[a] Already parasitized with *Entamœba coli* at the time of feeding *Amœba E*.
[b] This man subsequently became infected with *Entamœba histolytica* and developed a slight attack of dysentery on the 36th day after feeding.

cultures made to test the viability of the cysts showed a good growth of *Amœba H*. Cultures of this man's stools showed a growth of *Amœba H* on the first and second days after feeding, but never subsequently. Microscopic examinations of his stools were constantly negative. This man was under observation one year four months and eighteen days after this experiment began. No symptoms of dysentery developed.

Experiment XX.—Man 10, aged 45 years, had been under observation in the prison four years and eleven months. He gave a history of dysentery three years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen disclosed thickened bands along the sigmoid. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 3 Petri-plate cultures of *Amœba 13F*, mixed with magnesium oxide. This strain of *Amœba F* had been isolated in culture from a stool of an acute case of entamœbic dysentery in Manila. The cultures of this amœba ingested by this man contained encysted forms only. Transplant cultures made to test the viability of the cysts showed a luxuriant growth of the amœba. *Amœba F* was recovered in cultures from this man's stool from the first to the third day after feeding, but never subsequently. Microscopic examinations of his stools for amœboid organisms were constantly negative. This man was under observation five months after this experiment began. No symptoms of dysentery developed.

The protocols are summarized in Table I.

From the protocols and Table I it will be seen that, with the exception of species *A* and *C*, the specific amœba ingested in these experiments was in every case recovered in cultures on Musgrave and Clegg's medium from the stools of the man to whom they were fed on the first to the sixth day after ingestion, but never subsequently. Species *C* was ingested only once, and was not recovered in cultures. Three strains of species *A*, fed five times, were never recovered in cultures of the stools of the experimental men.

On the other hand, microscopic examination of the stools of these men were, with one apparent exception, always negative, although in many of these experiments the men have been under observation for over two years. The one exception to this result was in experiment IX, in which the man was already parasitized with *Entamœba coli* before ingesting a culture of *Amœba E*. *Amœba E* was recovered in cultures on the first and second days after feeding and not subsequently, but it was never found microscopically in the stools of this man. *Entamœba coli*, on the other hand, was never obtained in cultures, but it was constantly found microscopically in the stools of this man. Thus *Amœba E* behaved like the other amœbæ in the intestinal tract of man, and it was distinguished morphologically and biologically from *Entamœba coli* in this experiment.

In consequence of the failure in every case to find the amœbæ microscopically in the stools of the men who had ingested cultures of amœbæ and the ability to recover them in cultures only during the first few days after the ingestion, it is probable, as Werner (1908) and later one of us, Walker (1911), have concluded, that the cysts of the ingested amœbæ pass unchanged through the intestinal tract and find conditions suitable for development when the fæces are placed on the culture medium. In the case of experiments X and XVI, in which only motile amœbæ were ingested, the use of magnesium oxide to neutralize the acidity of the contents of the stomachs of the men may have favored the existence of the amœbæ in their passage through the intestinal tract of these men. It is also possible that the amœbæ became encysted (a protective reaction that takes place under any unfavorable conditions) in the intestines of these men. The failure to recover in cultures of the stools 3 strains of species *A*, fed five times, indicates that the cysts of this species are ordinarily incapable of withstanding passage through the human stomach.

Therefore, in consequence of our failure to parasitize men in 20 ingestion experiments with 13 strains of 8 species of amœbæ, we believe that the conclusion reached in the morphological study (Walker, 1911), that the cultivable amœbæ are not capable of living as parasites in the human intestine, is experimentally proved.

Following the feedings with the cultivable amœbæ, one man (experiment XIV) who had ingested a culture of *Amœba G*, isolated from a diarrhœal stool in Kansas, United States, outside of the endemic region, developed a slight attack of dysentery of two days' duration, thirty-five days after feeding. *Amœba G* ingested by this man was recovered in cultures on the first and second days after feeding and never afterward. It could never be found microscopically in the stools of this man. Two other men (experiments XV and XVI), who ingested the same strain of amœba, showed a similar behavior of the amœba, but did not develop dysentery. On the other hand, the amœboid organism found in the stools of the man in experiment XIV during the attack of dysentery belonged to a species and genus (*Entamœba histolytica*) distinct from the organism ingested by this man. It could not be cultivated on Musgrave and Clegg's medium, but was demonstrable microscopically in the stools during, and subsequent to, the attack of dysentery. Moreover, *Entamœba histolytica* from the stools of this man has been used to infect

other men in whom this entamœba maintained its characters of noncultivability and persistence microscopically in the stools and in some of whom dysentery has developed.

This case well illustrates the erroneous conclusions that have been drawn from experiments by investigators who have neglected to determine the species of amœboid organism fed to, and recovered from, the experimental animal. This man apparently either had a latent infection with *Entamœba histolytica* or had become infected with this entamœba after ingesting the culture of *Amœba G*. If the species of the amœboid organisms fed to this man, and that recovered in his stools during the attack of dysentery, had not been determined, and if the case had not been carefully followed with daily microscopic and cultural examinations, the conclusions would have been inevitable that *Amœba G*, cultivated from a diarrhœal stool in Kansas, was capable of producing entamœbic dysentery in man. As it is, we are in position to make the unqualified statement that *Amœba G* had nothing to do with the development of dysentery in this man; and, moreover, that *Amœba G* is not only not pathogenic, but that it is incapable of living as a parasite in the intestine of man.

In view of the fact that it has not been found possible to produce dysentery in man by 20 ingestion experiments made with 13 strains of 8 species of amœbæ cultivated from a variety of nonparasitic sources and from normal and dysenteric stools and that it has been demonstrated experimentally that none of these amœbæ are capable of living parasitically in the intestinal tract of man, the conclusion appears warranted that the *Amœbæ* play no part in the etiology of endemic tropical dysentery. The sound basis of this conclusion will be more evident when we consider the behavior of the *Entamœbæ* in the human intestine.

PART III. FEEDING EXPERIMENTS WITH ENTAMŒBA COLI

By Ernest Linwood Walker and Andrew Watson Sellards

It has been shown in a previous paper by one of us (Walker, 1911) that the amœboid organisms, living parasitically in the intestinal tract of man and other animals, differ morphologically and biologically from those found in water and soil, and occasionally cultivable from fæces, sufficiently to justify the establishment of the genus *Entamœba* by Cassagrandi and Barbagallo (1897) for the former species. Morphologically the parasitic *Entamœbæ* are differentiated from the nonparasitic *Amœbæ* by the absence of a contractile vacuole, by the eccentric instead of

central position occupied by the nucleus in the resting organism, by the peripheral instead of central arrangement of the chromatin in the nucleus, and by the presence of 4 or 8 nuclei, instead of a single nucleus, in the encysted stage; and biologically they are distinguished by their parasitic instead of saprozoic mode of life, by the occurrence of a reproductive process (schizogony) in the encysted stage, by their inability to propagate outside of the body of their host, and by not being cultivable on Musgrave and Clegg's medium (compare figs. 1 and 2 with figs. 3 to 8, Plate I).

Entamœba coli was first distinguished from another species, *Entamœba histolytica*, found in the intestinal tract of man by Schaudinn in 1903. This species was described by Schaudinn as follows. The entamœba shows no separation of the ectoplasm from the entoplasm in the resting stage. In the motile entamœba the ectoplasm is apparent in the hyaline pseudopodes, which are always less strongly refractive than the entoplasm. The nucleus is vesicular, spherical in the resting entamœba, and has a thick nuclear membrane. In the center of the nucleus of the vegetative entamœba are one or more small granules of plastin and chromatin. The chromatin is distributed as fine granules through the achromatic network of the nucleus, and appears to be collected particularly about the nuclear membrane. Multiplication takes place in the vegetative stage by simple division and by schizogony into 8 daughter entamœbæ. Cysts are developed, within which an autogamous sexual process takes place, followed by the development of 8 nuclei which give rise to 8 daughter entamœbæ when the cyst is ingested by a new host.

Schaudinn (1903) found *Entamœba coli* in 50 per cent of healthy persons in West Prussia, in 20 per cent at Berlin, and in 66 per cent of the population on the shores of the Adriatic Sea. Craig (1905) found 65 per cent of 200 American soldiers recruited from various parts of the United States parasitized with *Entamœba coli*. The occurrence of this species in the United States has been confirmed by Sistrunk (1911) who found it in the stools of 11 out of 145 patients suffering from diseases other than dysentery at Rochester, Minnesota; by Stiles (1911) who has observed it in North Carolina; and by Rosenberger and Terrell (1913) who found entamœbæ in 112 out of 137 males and in 81 out of 141 females examined at Philadelphia. In none of these cases was there a history of diarrhœa or dysentery. Vedder (1906) found *Entamœba coli* in 50 per cent of healthy

American soldiers and in 72 per cent of Filipino scouts in the Philippine Islands. These figures for the Philippines have been confirmed by Craig and Ashburn who found 71 per cent of healthy American soldiers parasitized. Evidence of the wide distribution of *Entamœba coli* is further substantiated by McCarrison in India, by Wenyon (1908) in Khartum, by Elmassian (1909) in South America, by Whitmore (1911) in Manila and Saigon, by Prowazek (1911) in Samoa, and by Darling (1912) upon the Isthmus of Panama.

Recently several other entamœbæ of the *coli* type have been described as distinct species. Prowazek (1911) found associated with *Entamœba coli* in human fæces in Suwaii and Saipipi an entamœba which he called *Entamœba williamsi.* This species is said to differ from *Entamœba coli* in the presence of "excretion crystals" in its cytoplasm, in its movements and feeding habits, in its peculiar chromidia formation, and in that it develops cysts containing 10 instead of 8 nuclei.

Beaurepaire Aragao (1912) describes an entamœba from the stools of a child in Brazil which is said to differ from *Entamœba coli* by the presence of a bundle of "siderophile substance," sometimes double, which divides the cyst into two approximately equal parts. The author designates this entamœba by the name *Entamœba brasiliensis.*

Prowazek (1912) found another entamœba associated with *Entamœba coli* in the stools of a woman in Sawaii, which he considers a distinct species, and which he named *Entamœba hartmanni.* This entamœba is said to differ from *Entamœba coli* in its small size, in the variable size of its nuclei, and by the very characteristic minute chromidia in the cytoplasm.

It is noteworthy that 2 out of these 3 so-called species have been found but once, that 2 out of the 3 have been found associated in the same patient with typical *Entamœba coli,* and that all 3 were found in regions where infections with *Entamœba coli* are common. The differential characters of these so-called new species consist chiefly in differences in size and cytoplasmic contents, variable size of the nuclei, and the number of nuclei in the cyst. Such differences are not uncommon mingled with typical *coli* forms. Abnormally large or small *coli* are frequently met with, and within certain limits the size of the nuclei and the cytoplasmic contents of entamœbæ are exceedingly variable. These variations represent chiefly metabolic and reproductive, but sometimes degenerative, changes in the entamœba. The number of nuclei in the cysts of *Entamœba coli* is also subject

to variation. While 8 is the usual number, individual cysts containing from 9 to 16, usually mingled with the 8 nuclear cysts, are not uncommon. Therefore, it is believed that the entamœba found so commonly in the stools of healthy persons in tropical and subtropical countries is of one species, *Entamœba coli* Schaudinn. It is unquestionably the common species in the Philippine Islands.

This entamœba is distinguished from *Entamœba histolytica*, to be considered in part IV, by its porcelaneous appearance; greater refractiveness; more sluggish motility; the possession of a nucleus that is distinctly visible in the living entamœba and contains a relatively large amount of chromatin; by the development of cysts that are larger, more refractive, and contain 8 or more, instead of 4, nuclei; and by their frequent occurrence in the stools of healthy persons (compare figs. 3 and 4 with figs. 5 to 8, Plate I).

Among the numerous experimental infections with entamœbæ that have been attempted by various authors, the following were made with entamœbæ identified as *Entamœba coli.*

Schaudinn (1903) experimented with kittens and also parasitized himself on two occasions by swallowing cysts of *Entamœba coli.* In both of his own infections the entamœbæ were said to have persisted in his stools two months. None of these experimental infections of himself or of kittens were followed by the development of dysenteric symptoms.

Craig (1905) made a large number of experiments on the pathogenicity of *Entamœba coli,* using kittens to which the fæces containing the entamœbæ were fed in milk or injected rectally. In some cases the injections were repeated from five to ten times in the same cat. In none of these experimental animals did symptoms of diarrhœa or dysentery develop.

Wenyon (1912) attempted to infect cats with fæces containing cysts of *Entamœba coli* which were administered *per œsophagus* and *per rectum* in large doses. In no case were these experimental infections followed by dysenteric symptoms. These same animals were subsequently infected with "*Entamœba tetragena*" and developed entamœbic dysentery.

Craig (1913) quotes a personal communication from Creighton Wellman. He attempted to infect 5 kittens by injecting rectally 4 to 5 cubic centimeters of fæces containing *Entamœba coli.* None of the animals developed symptoms of dysentery, and no dysenteric lesions were found in their intestines at necropsy about one month after injection.

In our series of experiments 20 men have ingested *Entamœba coli.* The men employed were carefully examined before use with reference to previous attacks of dysentery and to present parasitization with amœboid organisms. A few of the men gave a history of dysentery at some earlier period of their lives. Cultures on Musgrave and Clegg's medium and microscopic

examinations, both before and after a purgative, were made of their stools, and all men showing amœboid organisms after either method of examination were excluded.

Five distinct strains of *Entamœba coli* were employed as follows:

Strain A, from a healthy Filipino.
Strain B, from a Filipino suffering from an epithelioma of the jaw.
Strain C, from a healthy Filipino.
Strain D, from a Filipina, suffering from lobar pneumonia.
Strain E, from a healthy American.

Entamœba coli was fed for the most part in the encysted stage, since there are reasons for believing it to be the stage naturally infective and it was the purpose of these experiments to secure as high a percentage of parasitization as possible in order to determine the pathogenesis of this species.

The entamœbæ were mixed with powdered starch or magnesium oxide, inclosed in gelatine capsules, and ingested by the men. The starch or magnesium oxide was used, as in the experiments with the cultures of amœbæ, to absorb the excess of moisture that might dissolve the gelatine capsule and to facilitate the ingestion of the material. The magnesium oxide, when used, served also to neutralize the acidity of the contents of the stomach of the man. It is doubtful if this be necessary to secure parasitization with the entamœbæ, since the action of the gastric juices probably plays an important part in the dissolution of the cyst wall of the parasite. However, it was employed in certain of these experiments to correspond with the feeding experiments with the cultures of amœbæ. The percentage of parasitizations with *Entamœba coli* did not appear to be materially affected by its use.

Following the ingestion of material containing *Entamœba coli*, the stools of the men were examined daily, culturally and microscopically, for amœboid organism until it was determined that the ingested entamœbæ had parasitized or failed to parasitize the man, and thereafter at frequent intervals. The men were examined clinically and physically whenever conditions seemed to warrant.

A complete protocol is given of each man in order to put on record the details of these experiments.

Experiment XXI.—Man 11, aged 26 years, had been under observation in the prison for six years and two months. He had not been used for previous experiments. He gave a history of one attack of dysentery eight years ago. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were

negative. He ingested cysts of *Entamœba coli*, strain *A*, mixed with magnesium oxide. This man has been under observation two years since the experiment began. Following the feeding, cultures of this man's stools were constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the fourth day after feeding and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXII.—Man 6, aged 27 years, had been under observation in the prison for five years and seven months. He gave a history of a bloody mucous dysentery of four months' duration two years ago. He had been used previously for 1 feeding experiment with a culture of amœbæ, which was followed by negative results (experiment XVIII, part II). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested encysted and motile *Entamœba coli*, strain *A*, mixed with magnesium oxide. This man has been under observation one year and four months since this experiment began. Following the ingestion, cultures of this man's stools were constantly negative for amœboid organisms. Microscopic examinations showed *Entamœba coli* in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXIII.—Man 8, aged 57 years, had been under observation in the prison seven years and nine months. He gave a history of 1 attack of dysentery of one month's duration eight years ago. He had been used for 1 feeding experiment with a culture of amœbæ with negative results (experiment VIII, part II). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *A*, which had been kept at room temperature for ten days, mixed with powdered starch. This man has been under observation two years and five months since this experiment began. Cultures of his stools were constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the seventh day and more or less constantly ever since. No symptoms of dysentery have developed.

Experiment XXIV.—Man 12, aged 44 years, had been under observation in the prison for seven years and eight months. He had not been used for previous experiments. He had a negative dysenteric history. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *A*, which had been kept at room temperature ten days before use in this experiment, mixed with powdered starch. This man was under observation one year and ten months after this experiment began. Cultures of his stools on Musgrave and Clegg's medium were constantly negative for amœbæ. Microscopic examinations showed *Entamœba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXV.—Man 13, aged 48 years, had been under observation in the prison seven years and four months. He had not been used previously for experiments. He gave a history of 1 attack of dysentery of one week's duration twenty years ago. Physical examination of his abdomen showed a sigmoid that was palpable, firm, and smooth. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *A*, which had been kept at room temperature for ten days, mixed with powdered starch. This

man has been under observation two years and five months since this experiment began. Cultures of his stools on Musgrave and Clegg's medium for amœbæ have been constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXVI.—Man 14, aged 24 years, had been under observation in the prison seven years and seven months. He had not been used previously for experiments. History and physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *B*, which had been kept at room temperature two days, mixed with powdered starch. This man was under observation one year and three months after this experiment began. Cultures of his stools on Musgrave and Clegg's medium were constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the first day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXVII.—Man 15, aged 25 years, had been under observation in the prison seven years and four months. He had not been used previously for experiments. His history with reference to dysentery was negative. Physical examination of his abdomen was not made. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *B*, which had been kept at room temperature for two days, mixed with powdered starch. This man has been under observation one year and eight months since this experiment began. Cultures of his stools on Musgrave and Clegg's medium were uniformly negative. Microscopic examinations showed *Entamœba coli* in his stools on the eleventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXVIII.—Man 16, aged 26 years, had been under observation in the prison six years and five months. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested encysted *Entamœba coli*, strain *C*, mixed with magnesium oxide. Since this experiment began this man has been under observation one year and six months. Cultures of his stools on Musgrave and Clegg's medium have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the first day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXIX.—Man 17, aged 30 years, had been under observation in the prison five years and five months. He had suffered from 2 attacks of entamœbic dysentery four years and seven months and one year and five months previously, respectively. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *C*, mixed with magnesium oxide. This man was under observation four months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examinations of his stools were also negative; that is, this man failed to become parasitized with *Entamœba coli*. No symptoms of dysentery developed.

Experiment XXX.—Man 18, aged 32 years, had been under observation in the prison five years and two months. He had not been used for previous

experiments. His history with reference to dysentery was negative. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *C*, mixed with magnesium oxide. This man was under observation four months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery developed.

Experiment XXXI.—Man 19, aged 30 years, had been under observation in the prison six years and four months. He had not been used for previous experiments. His history with reference to dysentery was negative. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *C*, mixed with magnesium oxide. This man has been under observation one year and six months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examination of his stools for entamœbæ have also been negative; that is, this man failed to become parasitized with *Entamœba coli*. No symptoms of dysentery have developed.

Experiment XXXII.—Man 20, aged 47 years, had been under observation in the prison for four years and one month. He had not been used previously for experiments. He had a negative dysentery history. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *D*, mixed with magnesium oxide. This man has been under observation one year and three months since this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the sixth day and thereafter. No symptoms of dysentery have developed.

Experiment XXXIII.—Man 21, aged 30 years, had been under observation in the prison five years and one month. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested encysted *Entamœba coli*, strain *D*, mixed with magnesium oxide. This man has been under observation one year and three and one-half months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the eighth day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXXIV.—Man 22, aged 31 years, had been under observation in the prison five years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *D*, mixed with magnesium oxide. This man was under observation ten months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the eighth day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXXV.—Man 23, aged 38 years, had been under observation

in the prison five years and five months. He had not been used previously for experiments. He had a negative dysenteric history. Physical examination of his abdomen was not made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man was under observation eight months after this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXXVI.—Man 24, aged 25 years, had been under observation in the prison three years and nine months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXXVII.—Man 25, aged 26 years, had been under observation in the prison four years and one month. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man was under observation eight months after this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XXXVIII.—Man 26, aged 19 years, had been under observation in the prison two years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations of his stools were also negative; that is, this man failed to become parasitized with *Entamœba coli*. No symptoms of dysentery have developed.

Experiment XXXIX.—Man 27, aged 40 years, has been under observation in the prison seven years and one month. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed *Entamœba coli* in his stools on the second

TABLE II.—*Feeding experiments with Entamœba coli.*

Experiment No.	Records of men previous to experiment.							Sources and description of material fed.						Results of feeding experiments.			
	No.	Age.	Time under observation in prison.	History with reference to dysentery.	Physical examination of abdomen.	Used for previous feeding experiments.	Microscopic examination of stools.	Culture of stools on Musgrave and Clegg's medium.	Source of material.	Clinical history of person from whom material was obtained.	Strain of *Entamœba coli*.	Stage of development of the entamœbæ.	Quantity of material fed.	Material fed with—	Time under observation after feeding.	Culture of stools on Musgrave and Clegg's medium.	Microscopic examination of stools.	Dysentery.
		Yrs.	*Yrs. mos.*												*Yrs. mos. days.*			
XXI	12	26	6 2	1 attack of dysentery 8 years ago	Negative	None	Negative	Negative	Filipino	Healthy	*A*	Encysted and motile	3 gelatine capsules	Magnesium oxide	2 0 8	Negative	*Entamœba coli* on fourth day and thereafter	Negative.
XXII	6	27	5 7	Bloody mucous stools 2 years ago	do	Culture of *Amœba RA*, 25 days previously with negative results.	do	do	do	do	*A*	do	do	do	1 4 9	do	*Entamœba coli* on seventh day and thereafter	Do.
XXIII	8	37	7 9	1 attack of dysentery 8 years ago	do	Culture of *Amœba SB*, 16 days previously with negative results.	do	do	do	do	*A*	Encysted	5 gelatine capsules	Starch	2 5 0	do	do	Do.
XXIV	12	44	7 8	Negative	do	None	do	do	do	do	*A*	do	do	do	1 10 20	do	*Entamœba coli* on second day and thereafter	Do.
XXV	13	48	7 4	1 attack of dysentery 20 years ago	Sigmoid palpable, firm, and smooth.	do	do	do	do	do	*A*	do	do	do	2 5 0	do	*Entamœba coli* on seventh day and thereafter	Do.
XXVI	14	24	7 7	Negative	Negative	do	do	do	do	Epithelioma of lower jaw	*B*	do	1 gelatine capsule	do	1 3 0	do	*Entamœba coli* on first day and thereafter	Do.
XXVII	15	25	7 4	do	None	do	do	do	do	do	*B*	do	do	do	1 8 0	do	*Entamœba coli* on eleventh day and thereafter	Do.
XXVIII	16	26	6 5	do	do	do	do	do	do	Healthy	*C*	do	do	do	1 6 0	do	*Entamœba coli* on first day and thereafter	Do.
XXIX	17	30	5 5	Entamœbic dysentery 4 years and 7 months and 1 year and 5 months ago.	do	do	do	do	do	do	*C*	do	do	Magnesium oxide	0 4 8	do	Negative; did not become parasitized	Do.
XXX	18	32	5 2	Negative	do	do	do	do	do	do	*C*	do	do	do	0 4 0	do	*Entamœba coli* on second day and thereafter	Do.
XXXI	19	30	6 4	do	do	do	do	do	do	do	*C*	do	do	do	1 6 0	do	Negative; did not become parasitized	Do.
XXXII	20	47	4 1	do	do	do	do	do	Filipina	Lobar pneumonia	*D*	do	2 gelatine capsules	do	1 3 14	do	*Entamœba coli* on sixth day and thereafter	Do.
XXXIII	21	30	5 11	do	do	do	do	do	do	do	*D*	do	do	do	1 3 15	do	*Entamœba coli* on eighth day and thereafter	Do.
XXXIV	22	31	5 11	do	do	do	do	do	do	do	*D*	do	do	do	0 10 4	do	do	Do.
XXXV	23	38	5 5	do	do	do	do	do	American	Healthy	*F*	do	1 gelatine capsule	Starch	0 8 0	do	*Entamœba coli* on second day and thereafter	Do.
XXXVI	24	25	3 9	do	do	do	do	do	do	do	*F*	do	do	do	0 8 0	do	*Entamœba coli* on seventh day and thereafter	Do.
XXXVII	25	26	4 1	do	do	do	do	do	do	do	*E*	do	do	do	0 8 0	do	*Entamœba coli* on first day and thereafter	Do.
XXXVIII	26	19	2 11	do	do	do	do	do	do	do	*E*	do	do	do	0 8 0	do	Negative; did not become parasitized	Do.
XXXIX	27	40	7 1	do	do	do	do	do	do	do	*E*	do	do	do	0 8 0	do	*Entamœba coli* on second day and thereafter	Do.
XL	28	30	6 11	do	do	do	do	do	do	do	*E*	do	do	do	0 8 0	do	*Entamœba coli* on fourth day and thereafter	Do.

day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XL.—Man 28, aged 30 years, had been under observation in the prison six years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba coli*, strain *E*, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamœba coli* in his stools on the second day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

The protocols of these experiments with *Entamœba coli* are summarized in Table II.

The results of the experiments with *Entamœba coli* present a striking contrast to those obtained after feeding cultures of amœbæ (part II). The *Amœbæ* were, with the exception of 2 species, always recovered in cultures on Musgrave and Clegg's medium from the stools of the men the first few days after feeding; while similar cultures of the stools of men who had ingested *Entamœba coli* have been invariably negative. Furthermore, the *Amœbæ* could never be found microscopically in the stools of the men who had ingested them; on the other hand, *Entamœba coli* has been found microscopically, after a short incubation period, in the stools of every man who became parasitized (88 per cent of the men), and the entamœbæ have persisted in the stools for an indefinite time.

Of the 20 men who ingested *Entamœba coli*, 17 became parasitized after the first feeding and 3, who did not become parasitized, were reserved as controls. Of the 12 men who ingested the entamœbæ mixed with powdered starch and of the 8 men who ingested the entamœbæ mixed with magnesium oxide, 11 and 6, respectively, became parasitized.

The incubation period of *Entamœba coli*, that is, the time elapsing from the day of ingestion to the appearance of the entamœbæ in the stools of the men, as determined by the 20 experiments, varies from one to eleven days, with an average of 4.7 days.

None of the 17 men experimentally parasitized with *Entamœba coli* nor the 3 nonparasitized controls have developed any symptoms of dysentery, although some of these have been under observation for two years and five months.

From the uniform results obtained in these experiments with

Entamœba coli, we believe that we are justified in the conclusions that *Entamœba coli*, unlike the *Amœbæ*, is an obligatory parasite and cannot be cultivated on Musgrave and Clegg's medium, and that it is nonpathogenic and consequently plays no rôle in the etiology of entamœbic dysentery.

PART IV. FEEDING EXPERIMENT WITH "ENTAMŒBA TETRAGENA" AND ENTAMŒBA HISTOLYTICA

By Ernest Linwood Walker

Of the identified species of *Entamœba*, 3, *Entamœba histolytica* Schaudinn, *"Entamœba tetragena"* Viereck, and *"Entamœba minuta"* Elmassian, have been found associated with endemic tropical dysentery and have been definitely implicated in the etiology of this disease.

Entamœba histolytica was first described by Schaudinn in cases of dysentery from Egypt, China, and Siam in 1903. It is distinguished, according to this author, by its morphology and its developmental cycle. This entamœba possesses a distinct, refractive ectoplasm and a granular, vacuolated entoplasm. The nucleus is scarcely visible in the living entamœba, is eccentric in position, is frequently deformed by the movements of the entamœba, possesses no limiting membrane, and is poor in chromatin, which is arranged chiefly about the periphery. *Entamœba histolytica*, according to Schaudinn, does not become encysted *in toto* and undergo schizogony within the cyst, as does *Entamœba coli;* instead, there are developed small peripheral buds, containing chromidia derived from the nucleus, which are constricted off from the parent entamœba and become surrounded by a resistant capsule.

Of the numerous feeding experiments that have been made upon animals, in the following only has *Entamœba histolytica* been specifically identified:

Schaudinn (1903) produced a typical dysentery in 3 cats with characteristic lesions and entamœbæ in the bloody mucous stool by feeding a dysenteric stool containing *Entamœba histolytica*.

Craig (1905) produced dysentery in 50 per cent of the kittens injected rectally and in 66 per cent of 8 kittens fed dysenteric stools containing *Entamœba histolytica*. At necropsy, typical lesions were observed, and on section *Entamœba histolytica* was found in the tissues.

Shirota (1912) was able to produce a dysentery, having the same lesions as in man and with *Entamœba histolytica* in the stools and lesions, by introducing the stools of a human dysenteric patient into the rectum of young cats. The bacteria isolated in cultures from the same dysenteric fæces, when introduced into the rectum of other kittens, produced no clinical symptoms or pathological changes.

Wenyon (1912) has recently conducted the most extensive and successful experimental infections of animals with dysenteric fæces containing *Entamœba histolytica* yet attempted. He introduced the material containing the entamœbæ *per œsophagus* and *per rectum* into cats. Of 14 experiments on 12 cats, 8 were followed by the development of acute dysentery with typical ulcerations and entamœbæ in their stools, and one cat developed, in addition to the dysentery, 4 abscesses of the liver. He was able to pass the infection successively through 4 cats when it was lost. This author was further able to study the invasion of the tissues of infected cats. "The amœbæ make their way to the bottom of the tubular glands in the large intestine. Then they multiply and by pressure of their numbers or by the exertion of their pseudopodia, and probably through some toxic substance excreted by them, the lining cells are weakened and separated and the amœbæ pass into the connective tissue beneath."

Craig (1913) quotes a personal communication from Creighton Welman giving the data of 5 infection experiments of kittens with *Entamœba histolytica* performed in 1910. Two of the kittens received rectal injections of fæces containing the entamœbæ and 3 were fed the same material. Four out of the 5 kittens developed dysenteric symptoms or showed the characteristic lesions at necropsy.

"Entamœba tetragena" was first described by Viereck in 1907 in 2 cases of dysentery from India. Shortly afterward it was described under the name of *"Entamœba africana"* by Hartmann (1908), in cases of dysentery from Southwest Africa and South America. Subsequently it has been observed by several investigators in dysenteries in different parts of the Tropics, and it is stated by Whitmore (1911) and Hartmann (1912) to be the most common pathogenic species in the Philippine Islands. This entamœba is distinguished from *Entamœba histolytica,* according to Hartmann (1908 and 1912), by the nuclear structure of the vegetative stage, by its reproduction, and by the structure of its cysts. The nucleus of the vegetative stage, unlike that of *Entamœba histolytica,* is distinctly visible in the living entamœba, has a double-contoured membrane, and is rich in chromatin which has a characteristic arrangement. There is a pheripheral layer of chromatin and a central karyosome. This karyosome undergoes cyclical changes, but in its most characteristic stage consists of a central granule, the "centriol," which is surrounded by a clear halo bounded by a layer of chromatin granules. *"Entamœba tetragena,"* unlike *Entamœba histolytica,* becomes encysted *in toto* and undergoes schizogony within the cyst, but differs from *Entamœba coli* in that only 4 merozoites are formed. The unincubated cyst, therefore, contains 4 instead of 8 nuclei.

The following experimental infection of animals have been attempted with entamœbæ identified as *"Entamœba tetragena":*

Hartmann (1908) states that this species is as a rule less pathogenic for cats than *Entamœba histolytica.* Of 3 cats used in his experiments, 1 did not develop dysentery, 1 showed after from eight to ten days slightly bloody stools for a few days only, and the third developed a more severe dysentery and died three weeks after infection.

Werner (1909) experimented with 5 strains of *"Entamœba tetragena."* Two of these strains when injected into the rectum of cats gave rise to no infection, the other 3 strains produced a dysentery in the experimental animals. One of these latter strains was passed through 5, another through 3 cats, and a third through 1 cat, after which their virulence was lost. The period of incubation is given as from five to twelve days with an average of seven and one-half days. The duration of the disease in cats was from eight to thirty-two days. Of the successfully infected cats, 6 died. These showed typical ulcerations of the large intestine, and one had an abscess of the liver.

Franchini (1911) introduced into the rectum of a healthy monkey, which had been under observation in the laboratory more than one year, fæces from a case of tropical dysentery containing blood, mucus, and numerous *"Entamœba tetragena."* Three injections of this material were made, on February 10, 18, and 20, respectively. On May 10 the monkey developed dysentery with numerous entamœbæ in his stools. At necropsy the cæcum was found to contain one large and numerous small ulcers, and the rest of the intestine showed more or less colitis. *"Entamœba tetragena"* was found in the intestinal contents and in sections of the large intestine.

Darling (1912) fed 2 kittens with the cysts of *"Entamœba tetragena"* from a case of entamœbic dysentery. On the twelfth day both kittens had prolapse of the rectum following intussusception and entamœbic enteritis. He was unable, in numerous feeding experiments with monkeys, dogs, and cats, to infect with the motile or trophozoite stage of this entamœba.

Craig (1913) quotes a personal communication from Dr. H. B. Fantham who had succeeded in producing dysentery in 1 of 2 kittens fed fæces containing *"Entamœba tetragena"* from an infection contracted in Algeria. The kitten died in three weeks, and ulcerations containing *"Entamœba tetragena"* were found in the intestine. All of his experiments by rectal injections of the material into kittens were negative.

"Entamœba minuta" was found by Elmassian (1909) in the stools of a case of recurrent dysentery in a European who had resided in Paraguay, South America. This *Entamœba* had, in the living organism, an indistinct nucleus like *Entamœba histolytica,* but in stained preparations the nucleus showed a heavy peripheral ring of chromatin like *Entamœba coli.* No distinction existed between ectoplasm and entoplasm, and its movements were sluggish. Small cysts, 12 to 14 microns in diameter, were developed which contained 4 nuclei. The author considers this species to be pathogenic, but no experiments were undertaken to prove its pathogenicity.

In a previous paper (Walker, 1911) I have expressed the opinion, based upon morphological evidence, that *"Entamœba tetragena"* Viereck is identical with *Entamœba histolytica* Schau-

dinn and that the life cycle of *Entamœba histolytica* includes the development of "tetragena" cysts.

Wenyon (1912), Darling (1912), and Hartmann (1912) have subsequently come to the same conclusion, although Darling and Hartmann persist in calling the species *"Entamœba tetragena."* However, on the basis of priority, *Entamœba histolytica* Schaudinn must remain the valid name of this species. Craig (1913) still maintains that *Entamœba histolytica* and *"Entamœba tetragena"* are distinct species.[11]

The view that we are here dealing with but one species has received further support from observation of the morphological changes that take place in *Entamœba histolytica* during the course of the disease in experimentally infected men. There has been found to exist a more or less definite series of morphological changes in the entamœbæ that are found in the stools which appear to be correlated with the clinical symptoms in the host. Men fed *Entamœba histolytica* show *"tetragena"* cysts in their stools, after a short incubation period, and these cysts persist so long as the stools of the parasitized individual remain formed. When the stools become soft or diarrhœal, the *"tetragena"* cysts are replaced by postencysted or preëncysted entamœba which are small and inactive and have a nucleus more or less rich in chromatin. These forms correspond to Elmassian's *"Entamœba minuta."* If chronic dysentery with fæcal stools mixed with mucus and blood develops, larger and more active forms appear which still contain nuclei rich in chromatin and many of which show the karyosome structure characteristic of *"Entamœba tetragena."* In acute attacks of dysentery, in which mucus and blood practically free from fæces are passed, these forms are largely replaced by entamœbæ having an indistinct nucleus that contains a minimum amount of chromatin, which is characteristic of *Entamœba histolytica* Schaudinn. In untreated cases that recover spontaneously from the attack of dysentery, this series of morphological changes is repeated in the inverse order, ending with the reappearance of *"tetragena"* cysts in the formed stools of the convalescent individual. These changes in the morphology of *Entamœba histolytica*, which are connected with the developmental cycle of the organism, probably

[11] Since this paper was written, Craig (1913[a] and 1913[b]) has changed his opinion and now agrees that *Entamœba tetragena* Viereck is identical with *Entamœba histolytica* Schaudinn. He gives me full credit for being the first definitely to state the identity of these two species.

account for the several species of *Entamœba* that have been described by different observers in dysenteric stools.

An attempt has been made to obtain experimental evidence of the truth of these conclusions by the use in feeding experiments of either motile entamœbæ of the *histolytica* type or *"tetragena"* cysts only. The material for these feeding experiments was selected after a careful microscopic examination of both fresh and stained preparations.

Experiment 1.—Men 5 and 34 ingested motile and resting entamœbæ of the *histolytica* type only from a case of acute entamœbic dysentery. Both men became parasitized, and *"tetragena"* cysts appeared in the formed stools of these men on the fourth day after ingestion and have persisted ever since.

Experiment 2.—Man 2 ingested *"tetragena"* cysts from a convalescent case of entamœbic dysentery. This man became parasitized, and *"tetragena"* cysts appeared in his stools on the second day after the ingestion of the entamœbæ. These cysts persisted in the stools of this man until the twentieth day, when acute dysentery developed with typical motile *histolytica* only in his bloody mucous stools. The patient was given treatment, and recovered from the attack of dysentery on the thirtieth day, when *"tetragena"* cysts reappeared in his normal stools. On the sixtieth day he suffered a relapse with motile *histolytica* only in his bloody mucous stools. Treatment again relieved the dysenteric symptoms, but *"tetragena"* cysts soon reappeared and have been found more or less constantly ever since in his stools.

Therefore, in these experiments it has been possible to obtain (*a*) *"tetragena"* cysts in the stools of men fed motile *histolytica* only, (*b*) motile *histolytica* in the stools of a man fed exclusively with *"tetragena"* cysts, and (*c*) an alternation of motile *histolytica* and *"tetragena"* cysts in the stools of a man having recurrent attack of entamœbic dysentery. The conclusions, therefore, appear warranted that *Entamœba tetragena* Viereck is identical with *Entamœba histolytica* Schaudinn, that *"tetragena"* cysts are produced in the life cycle of *Entamœba histolytica*, and that *Entamœba minuta* Elmassian is the preëncysted stage of *Entamœba histolytica*.

Entamœba histolytica Schaudinn (which includes *"Entamœba tetragena"* Viereck and *"Entamœba minuta"* Elmassian) is distinguished from *Entamœba coli*, previously considered, by a less refractive and more hyaline appearance; by a more active motility; by an indistinct nucleus that is relatively poor in chromatin; by cysts that are smaller and less refractive, which usually contain one or more refractive bodies that stain with chromatin stains and are designated by Hartmann (1912) as "Chromidialkörper," and 4 instead of 8 nuclei; and by their

less frequent occurrence in the stools of healthy persons and their constant presence in the stools of cases of endemic tropical dysentery (compare figs. 5 to 8 with figs. 3 and 4, Plate I).

In the following series of experiments 20 men have ingested material containing *Entamœba histolytica.* Four of these men had a history of attacks of dysentery from six to sixteen years previously; the other 16 had negative dysenteric histories. Four of the men had been used previously for ingestion experiments with cultures of amœbæ with negative results. All of the men were free from dysenteric symptoms, and their stools were proved to be free from amœboid organisms by cultures on Musgrave and Clegg's medium and by microscopic examinations before being used for these experiments.

The entamœbæ ingested by these men were from 7 different sources and represented 4 distinct strains of *Entamœba histolytica.* The history of these strains will be given in connection with the protocols of the experiments.

The material containing *Entamœba histolytica,* as in the case of the *Amœbæ* and *Entamœba coli,* was mixed with powdered starch or magnesium oxide, inclosed in a gelatine capsule, and ingested by the experimental men. In cases where motile entamœbæ were ingested and it was consequently undesirable to absorb the moisture of the material, the infectious material was inclosed in a small gelatine capsule and this inclosed in a larger gelatine capsule containing magnesium oxide. The use of magnesium oxide to neutralize the acidity of the contents of the stomach in the experiments with *Entamœba histolytica* was in order to secure parasitization with the motile entamœbæ and to insure infection with any possibly pathogenic microörganisms that might be associated with the entamœbæ and be the primary etiologic agent in the production of dysentery, especially in the control cases that did not become parasitized with *Entamœba histolytica.*

Cultures and microscopic examinations were made daily of the stools of the men after ingesting the infectious material until parasitization or nonparasitization with *Entamœba histolytica* was determined, and thereafter at frequent intervals. In every case the species of amœboid organism found by either method of examination was carefully determined. Clinical symptoms of dysentery were carefully watched for, and men who developed dysentery received ipecac treatment as soon as the diagnosis of entamœbic dysentery was clinically and microscopically established. Treatment of the cases of dysentery was

controlled by microscopic examination of the stools until each man was cured.

Complete protocols of each man are given in order to put on record the details of these experiments.

ENTAMŒBA HISTOLYTICA, STRAIN A, FIRST PASSAGE

Strain *A* of *Entamœba histolytica* was from a man convalescent from a slight attack of entamœbic dysentery of two days' duration. This man had been convalescent from fifty-nine to one hundred sixty-one days when the entamœbæ from his stools were used for these feeding experiments, and he has not subsequently suffered a relapse of the dysentery. His formed stools contained many cysts of *Entamœba histolytica.* This strain was ingested by 3 men and carried by subsequent passages through 9 other men.

Experiment XLI.—Man 2, aged 40 years, had been under observation in the prison five years and one month. He had a negative dysenteric history. He had been used previously for 2 feeding experiments with cultures of amœbæ, ninety-six and seventeen days previously, both of which were negative (part II, experiments VII and V). Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, mixed with magnesium oxide. This man received a saline purgative by mistake in the evening of the day that he ingested the infectious material. He became parasitized with *Entamœba histolytica*, the encysted entamœbæ appearing in his stools on the eleventh day. Cultures of his stools on Musgrave and Clegg's medium were negative. On the twentieth day he developed an attack of dysentery with bloody mucous stools containing motile *Entamœba histolytica*, many of which were filled with red blood corpuscles. Physical examination disclosed pain over the abdomen, no tenderness except over the sigmoid and cæcum, sigmoid not palpable, and liver dulness normal. He was put on ipecac treatment, and on the thirtieth day all symptoms of dysentery had disappeared. Encysted *Entamœba histolytica*, however, continued to be present in his stools. On the seventieth day there was a relapse of the dysentery with motile entamœbæ in the bloody mucous stools. Cultures of his stools on Musgrave and Clegg's medium were again negative. The patient was again put on ipecac treatment, the symptoms soon abated, and, up to the present time, he has suffered no further relapse, but encysted entamœbæ soon reappeared in his stools and have persisted ever since. He has been under observation for one year since the beginning of this experiment.

Experiment XLII.—Man 29, aged 35 years, had been under observation in the prison for seven years and ten months. He had not been used previously for experiments, and had no history of dysentery. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, mixed with magnesium oxide. Following the ingestion, both cultures and microscopic examination of the stools of man 2 were negative. Therefore, this man failed to

become parasitized with *Entamœba histolytica*. In order to determine whether this man possessed a relative or an absolute immunity, he ingested cysts of *Entamœba histolytica* a second time on the seventy-sixth day after the first. The infectious material was from the same "carrier" as the first ingestion, now convalescent one hundred thirty-six days, but still showing encysted *Entamœba histolytica* in his stools, and was mixed with magnesium oxide. Following this ingestion experiment, cultures of his stools were negative. Microscopic examination showed many small *Entamœba histolytica* in his stools on the seventy-ninth day, or three days after this second feeding, but subsequent examinations were negative. The entamœbæ evidently had failed again to establish themselves permanently as parasites in the intestine of this man. On the one hundred second day he ingested for the third time entamœbæ from the same "carrier," now convalescent one hundred sixty-two days. Following this ingestion experiment, cultures of the stools of this man were negative for amœbæ. Microscopic examination showed encysted *Entamœba histolytica* on the one hundred seventh day, or the fifth day after this last ingestion, and the entamœbæ have persisted in his stools ever since. This man has been under observation nine months since the last ingestion experiment, and he has not shown any dysenteric symptoms.

Experiment XLIII.—Man 30, aged 25 years, had been under observation in the prison seven years and five months. He had a negative dysenteric history, and had not been used for previous experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, mixed with magnesium oxide. Following the ingestion, cultures of the stools of man 30 were negative for amœbæ. Microscopic examination of his stools showed encysted *Entamœba histolytica* on the day following the ingestion, and the entamœbæ have persisted in his stools ever since. On the ninety-fifth day a stool from this man was semifluid and greenish, contained mucus, a little blood, and many motile and resting *Entamœba histolytica*, some of which contained red blood corpuscles. No examination of his stools had been made for six days previously, and another stool was not obtained until the second day after this examination. At the former examination the stool was soft and no entamœbæ were found; at the latter examination the stool was formed and contained a few encysted entamœbæ. *Entamœba histolytica* had been found in his stools eight days previous to this slight attack of dysentery. Physical examination of this man was negative. He has been under observation one year since the beginning of the experiment. No relapse of the dysentery has occurred, but the entamœbæ have persisted in his stools.

ENTAMŒBA HISTOLYTICA, STRAIN A, SECOND PASSAGE, SERIES 1

In this series of experiments strain *A* of *Entamœba histolytica* had been passed from the original case, convalescent sixty days from an attack of spontaneous entamœbic dysentery, through man 2. Man 2 had been infected one hundred forty-four days previously with strain *A*, had developed an attack of entamœbic dysentery one hundred twenty-five days, with a relapse seventy-four days, previously (experiment XLI), and was now a "con-

valescent carrier," passing cysts of *Entamœba histolytica* in his formed stools.

Experiment XLIV.—Man 31, aged 36 years, had been under observation in the prison six years and nine months. He had a negative history for dysentery, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, second passage, series 1, mixed with magnesium oxide. Cultures of the stool of man 31 following the feeding were negative for amœbæ. Microscopic examination of his stool showed *Entamœba histolytica* on the fifth day. This man has been under observation one year since the experiment began. The entamœbæ have been present in the stools of this man up to the present time, but no symptoms of dysentery have developed.

Experiment XLV.—Man 32, aged 30 years, had been under observation in the prison four years and eight months. He had a negative history for dysentery, and had not been used for previous experiments. Physical examination of his abdomen and cultural and microscopic examination of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, second passage, series 1, mixed with magnesium oxide. Cultures of the stools of man 32 after the ingestion were negative. Microscopic examination of his stools showed *Entamœba histolytica* on the third day after ingestion and thereafter. This man was under observation five and one-third months after the experiment began. No dysenteric symptoms developed.

Experiment XLVI.—Man 33, aged 30 years, had been under observation in the prison two years and seven months. He had a negative dysenteric history, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. This man ingested cysts of *Entamœba histolytica*, strain *A*, second passage, series 1, mixed with magnesium oxide. Following the ingestion, cultures of the stools of man 33 were negative. *Entamœba histolytica* was found microscopically in his stools on the fifth day and thereafter. He has been under observation one year since the experiment began. No symptoms of dysentery have developed.

ENTAMŒBA HISTOLYTICA, STRAIN A, SECOND PASSAGE, SERIES 2

This series of experiments was conducted with strain *A* which had been passed through man 29 (experiment XLII), who had been parasitized with this strain of *Entamœba histolytica* one hundred twenty-one days previously, but who had not, and has not subsequently, developed dysentery. This series of experiments, therefore, was made with *Entamœba histolytica* from a "contact carrier."

Experiment XLVII.—Man 36, aged 45 years, had been under observation in the prison eight years and five months. He had a negative

dysenteric history, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examination of his stools for amœboid organisms were negative. This man ingested encysted *Entamœba histolytica*, strain *A*, second passage, series 2, mixed with powdered starch. Cultures of the stool of man 36 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed *Entamœba histolytica* on the fourth day after the ingestion. This man has been under observation one year since the experiment began. *Entamœba histolytica* has been found constantly in his stools, but no dysentery has developed.

Experiment XLVIII.—Man 7, aged 30 years, had been under observation in the prison five years and two months. This man gave a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had been used previously for the following experiments. Two hundred forty-six days previously he had ingested a culture of *Amœba 9F*. Result negative (part II, experiment XIII). Two hundred twenty-five days previously he had ingested a culture of *Amœba 8A*. Result negative (part II, experiment XI). Two hundred fifteen days previously he had ingested a culture of *Amœba 10G*. Result negative (part I, experiment XVI). One hundred fifty-seven days previously he had ingested a culture of *Amœba 8B*. Result negative (part II, experiment VI). Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *A*, second passage, series 2, mixed with powdered starch. Cultures of the stools of man 7 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed *Entamœba histolytica* on the fourth day after the ingestion. This man has been under observation one year since the experiment began. The entamœbæ have persisted in his stools, but no dysentery has developed.

ENTAMŒBA HISTOLYTICA, STRAIN A, THIRD PASSAGE

The history of strain *A* of *Entamœba histolytica* employed in this series of experiments is as follows. Coming originally from a man convalescent sixty days from a slight attack of entamœbic dysentery, it has been passed through men 29 and 36. Man 29 had been parasitized with this strain for one hundred sixty-one days, without the development of dysentery, when it was passed through man 36. Man 36 had been parasitized one hundred twenty-one days with the strain, without the development of dysentery, when it was used for the present series of experiments. Neither man 29 nor 36 has subsequently developed symptoms of dysentery although both have shown the cysts of *Entamœba histolytica* more or less constantly in their stools up to the present time. Therefore, this strain of entamœbæ had been passed from a "convalescent carrier" through 2 "contact carriers" before being employed in the present series of experiments.

Experiment XLIX.—Man 41, aged 50 years, had been under observation in the prison three years and seven months. He had a negative dysenteric history. No physical examination was made of his abdomen. He had not been used for previous experiments. His stools were negative, both culturally and microscopically, for amœboid organisms. He ingested cysts of *Entamœba histolytica*, strain *A*, third passage, mixed with magnesium oxide. Cultures of the stools of man 41 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed *Entamœba histolytica* on the first day after feeding and thereafter. This man developed dysentery on the fifty-seventh day with abdominal pain and bloody mucous stools containing motile *Entamœba histolytica*. The dysentery lasted two weeks. He was treated with ipecac, has recovered, and has had no relapses. *Entamœba histolytica* disappeared temporarily from this man's stools following the ipecac treatment, but reappeared shortly and has persisted ever since.

Experiment L.—Man 42, aged 30 years, had been under observation in the prison four years and eleven months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not been used previously for experiments. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested encysted *Entamœba histolytica*, strain *A*, third passage, mixed with magnesium oxide. Cultures of the stools of man 42 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed *Entamœba histolytica* on the fourth day and constantly thereafter. He has been under observation one year since the experiment began. No dysenteric symptoms have appeared up to the present time.

Experiment LI.—Man 43, aged 38 years, had been under observation in the prison three years and two months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not previously been used for experiments. His stools were negative, culturally and microscopically, for amœboid organisms. This man ingested encysted *Entamœba histolytica*, strain *A*, third passage, mixed with magnesium oxide. Cultures of the stools of man 43 after the ingestion were negative for entamœbæ. Microscopic examination of his stools have been constantly negative for entamœbæ, and he has been reserved as a non-parasitized control. He has been under observation one year since the experiment began. No dysentery has developed up to the present time.

Experiment LII.—Man 44, aged 27 years, had been under observation in the prison one year and three months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not been used for previous experiments. Cultures and microscopic examinations of his stools for amœboid organisms were negative. This man ingested encysted *Entamœba histolytica*, strain *A*, third passage, mixed with magnesium oxide. Cultures of the stools of this man after the ingestion were negative for amœbæ. Microscopic examinations of his stools up to the sixth day were negative for entamœbæ. By an oversight his stools were not examined again until the thirty-third day, when *Entamœba histolytica* was found, and has been found more or less constantly ever since, in his stools. This man has been under observation one year since the experiment began. He has shown no symptoms of dysentery up to the present time.

ENTAMŒBA HISTOLYTICA, STRAIN B

This strain of *Entamœba histolytica* was from a man suffering from an acute attack of entamœbic dysentery, whose dysenteric stools contained many motile entamœbæ. The strain was used in the following two experiments, and there were no subsequent passages of it.

Experiment LIII.—Man 5, aged 30 years, had been under observation in the prison for seven years and six months. He gave a history of a mucous dysentery of one month's duration seven years ago. He had been used for a feeding experiment with a culture of *Amœba 11G*, two hundred fifty-seven days previously (part II, experiment XVII). The result of this experiment was negative. At the time of the present experiment, physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested motile *Entamœba histolytica*, strain *B*. After the ingestion, cultures of the stools of man 5 were negative for amœbæ. Microscopic examinations of his stools were negative for several weeks, and he was considered as a nonparasitized control. However, on the forty-fourth day *Entamœba histolytica* was found in his stool and has persisted ever since. He has been under observation one year since the beginning of the experiment. No dysenteric symptoms have appeared.

Experiment LIV.—Man 34, aged 30 years, had been under observation in the prison six years. He had a negative dysenteric history, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested motile *Entamœba histolytica*, strain *B*. Following the ingestion, cultures of the stools of man 34 were negative for amœbæ. *Entamœba histolytica* was found microscopically in the stools of this man on the fourth day after the ingestion and has persisted ever since. This man was under observation nine months after the beginning of this experiment. No symptoms of dysentery developed.

ENTAMŒBA HISTOLYTICA, STRAIN C

Strain *C* of *Entamœba histolytica* was obtained post mortem from a fatal case of entamœbic liver abscess. The patient from whom these entamœbæ were obtained died at 11 o'clock in the morning, a necropsy was performed at 1.30, and the entamœbæ were ingested by men 3 and 35 at 2.30 in the afternoon. There were no subsequent passages of this strain.

Experiment LV.—Man 3, aged 31 years, had been under observation in the prison six years. He had a history of dysentery of one month's duration sixteen years ago. He had been previously used for the following experiments. Two hundred seventy-nine days previously he ingested a culture of *Amœba 1A*. Result negative (part II, experiment I). Two hundred sixty-seven days previously he had ingested a culture of *Amœba 1A*. Result negative (part II, experiment III). Two hundred forty-three days previously he had ingested a culture of *Amœba 10G*. Result negative (part II, experiment XV). Two hundred eight days previously

he had ingested a culture of *Amœba 5D*. Result negative (part II, experiment IX). Physical examination of his abdomen and cultural and microscopic examinations of the stools for amœboid organisms were negative at the time of the present experiment. This man ingested motile *Entamœba histolytica*, strain *C*, mixed with magnesium oxide. Following the ingestion, cultures of the stools of this man were negative for amœbæ. *Entamœba histolytica* was found microscopically in his stools on the day following the ingestion and has persisted in his stools ever since. This man was under observation two months after the experiment began. No symptoms of dysentery developed.

Experiment LVI.—Man 35, aged 30 years, had been under observation in the prison six years and nine months. He had a negative history for dysentery, and had not been used for previous feeding experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested motile *Entamœba histolytica*, strain *C*, mixed with magnesium oxide. This man has been under observation one year since the experiment began. Cultures and microscopic examination of his stools have been constantly negative for amœboid organisms. He has consequently been reserved as a nonparasitized control. This man has shown no dysenteric symptoms.

ENTAMŒBA HISTOLYTICA, STRAIN D

Strain *D* consisted of encysted entamœbæ from a naturally parasitized "contact carrier" of *Entamœba histolytica*. This woman had no history of entamœbic dysentery, but her stools contained a moderate number of encysted *Entamœba histolytica*. This strain was used in 4 experiments, and there were no subsequent passages of it.

Experiment LVII.—Man 37, aged 32 years, had been under observation in the prison seven years and eight months. He had a history of an acute dysentery four years previously. He had not been used for previous experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *D*, mixed with powdered starch. Following the ingestion, cultures of the stools of man 37 were negative. Microscopic examination of his stools showed *Entamœba histolytica* on the eighth day after feeding. This man was under observation eight months and seventeen days after the experiment began. The entamœbæ have been found constantly in his stools, but no symptoms of dysentery have developed.

Experiment LVIII.—Man 38, aged 37 years, had been under observation in the prison six years and eight months. Dysenteric history was negative, and he had not been used for previous experiments. No physical examination of his abdomen was made. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamœba histolytica*, strain *D*, mixed with powdered starch. Cultures of the stools of man 38 following the ingestion were negative. Microscopic examination of his stools showed *Entamœba histolytica* on the twenty-first day after the ingestion and more or less constantly ever since. This man has been under observation one year since the beginning of the experiment. No dysenteric symptoms have developed.

TABLE III.—*Feeding experiments with Entamœba histolytica.*

Experiment No.	Records of men previous to experiment.							Source and description of the material ingested.						Results of feeding experiments.		
	No.	Age.	Time under observation in prison.	History with reference to dysentery.	Physical examination of abdomen.	Used for previous feeding experiments.	Microscopic examinations of stools.	Culture of stools on Musgrave and Clegg's medium.	Source of material.	Clinical history of the patient from whom the material was obtained.	Strain of *Entamœba histolytica*.	Stage of development of entamœba.	Quantity of material fed.	Material mixed with—	Time under observation after feeding.	Culture on Musgrave and Clegg's medium.	Microscopic examination of stools.	Dysentery.
		Yrs.	*Yrs. mos.*												*Yrs. mos. days.*			
XLI	2	40	8 1	Negative	Negative	Culture of *Amœba 1C* 96 days previously with negative results; culture of *Amœba 2A* 17 days previously with negative results.	Negative	Negative	Man 1	Convalescent 59 days from a spontaneous attack of entamœbic dysentery. Stools formed.	*A*	Encysted	2 gelatine capsules	Magnesium oxide	1 0 0	Negative	*Entamœba histolytica* on second day after feeding and thereafter.	Entamœbic dysentery on twentieth day, relapse on the sixtieth day.
XLII	29	35	7 10	do	do	*Entamœba histolytica* 102 and 26 days previously. Results negative.	do	do	do	Convalescent 161 days from a spontaneous attack of entamœbic dysentery. Stools formed.	*A*	do	3 gelatine capsules	do	0 9 0	do	*Entamœba histolytica* on fifth day after feeding and thereafter.	Negative.
XLIII	30	25	7 6	do	do	None	do	do	do	do	*A*	do	do	do	1 0 0	do	*Entamœba histolytica* on second day after feeding and thereafter.	Slight attack of entamœbic dysentery on the ninety-fifth day.
XLIV	31	35	6 9	do	do	do	do	do	Man 2	Convalescent 84 days from experimental entamœbic dysentery. Stools formed.	*A*	Resting and encysted	1 gelatine capsule	do	1 0 0	do	*Entamœba histolytica* on fifth day after feeding and thereafter.	Negative.
XLV	32	30	4 8	do	do	do	do	do	do	do	*A*	do	2 gelatine capsules	do	0 5 10	do	*Entamœba histolytica* on third day after feeding and thereafter.	Do.
XLVI	33	30	2 7	do	do	do	do	do	do	do	*A*	do	do	do	1 0 0	do	*Entamœba histolytica* on fifth day after feeding and thereafter.	Do.
XLVII	36	45	8 5	do	do	do	do	do	Man 29	"Contact carrier," experimentally parasitized with *Entamœba histolytica* 121 days, first passage. Stools formed.	*A*	Encysted	1 gelatine capsule	Starch	1 0 0	do	*Entamœba histolytica* on fourth day after feeding and thereafter.	Do.
XLVIII	7	30	5 2	3 attacks of dysentery 6 years ago	do	Culture of *Amœba 8A* 255 days previously; culture of *Amœba 2F* 246 days previously; culture of *Amœba 10G* 215 days previously. Results all negative.	do	do	do	do	*A*	do	do	do	1 0 0	do	do	Do.
XLIX	41	50	3 7	Negative	None	None	do	do	Man 36	"Contact carrier," experimentally parasitized with *Entamœba histolytica*, second passage. Stools formed.	*A*	do	do	Magnesium oxide	0 6 0	do	*Entamœba histolytica* on first day after feeding and thereafter.	Entamœbic dysentery on the fifty-seventh day.
L	42	30	4 11	do	do	do	do	do	do	do	*A*	do	do	do	1 0 0	do	*Entamœba histolytica* on fourth day after feeding and thereafter.	Negative.
LI	43	38	3 2	do	do	do	do	do	do	do	*A*	do	do	do	1 0 0	do	Negative; did not become parasitized	Do.
LII	44	27	1 3	do	do	do	do	do	do	do	*A*	do	do	do	1 0 0	do	*Entamœba histolytica* on thirty-third day after feeding and thereafter.	Do.
LIII	5	30	7 6	Mucous dysentery 7 years ago	Negative	Culture of *Amœba 11G* 251 days previously with negative results.	do	do	Man 3649	Suffering from an acute attack of entamœbic dysentery, with bloody mucous stools.	*B*	Motile and resting	2 gelatine capsules	do	1 0 0	do	*Entamœba histolytica* on forty-fourth day after feeding and thereafter.	Do.
LIV	34	30	6 0	Negative	do	None	do	do	do	do	*B*	do	do	do	0 9 0	do	*Entamœba histolytica* on fourth day after feeding and thereafter.	Do.
LV	3	31	6 9	Dysentery 16 years ago	do	Culture of *Amœba 1A* 279 days previously with negative results; culture of *Amœba 10G* 267 days previously with negative results.	do	do	Woman 1	Dead from an entamœbic liver abscess. Material obtained post mortem from liver abscess.	*C*	do	do	do	0 2 0	do	*Entamœba histolytica* on first day after feeding and thereafter.	Do.
LVI	35	30	6 9	Negative	do	None	do	do	do	do	*C*	do	do	do	1 0 0	do	Negative; did not become parasitized	Do.
LVII	37	33	7 9	Acute dysentery 4 years ago	do	do	do	do	Woman 2	"Contact carrier," naturally parasitized with *Entamœba histolytica*. Stools formed.	*D*	Encysted	1 gelatine capsule	Starch	0 8 17	do	*Entamœba histolytica* on eighth day after feeding and thereafter.	Do.
LVIII	38	37	6 8	Negative	None	do	do	do	do	do	*D*	do	do	do	1 0 0	do	*Entamœba histolytica* on twenty-first day after feeding and thereafter.	Do.
LIX	40	25	8 8	do	do	do	do	do	do	do	*D*	do	do	do	1 0 0	do	*Entamœba histolytica* on eleventh day after feeding and thereafter.	Do.
LX	39	32	6 2	do	do	do	do	do	do	do	*D*	do	do	do	1 0 0	do	do	Slight attack of entamœbic dysentery on the eighty-seventh day.

Experiment LIX.—Man 40, aged 25 years, had been under observation in the prison eight years and eight months. He had a negative dysenteric history. No physical examination of his abdomen was made. He had not been used for previous experiments. Microscopic and cultural examinations of his stools were negative for amœboid organisms. He ingested cysts of *Entamœba histolytica*, strain *D*, mixed with powdered starch. Cultures of the stools of man 40 after the ingestion were negative. Microscopic examination of his stools showed *Entamœba histolytica* on the eleventh day after the experiment began and more or less constantly thereafter. Since then he has been under observation one year, but has had no symptoms of dysentery.

Experiment LX.—Man 39, aged 32 years, had been under observation in the prison six years and two months. He had a negative dysenteric history. No physical examination of his abdomen was made. He had not been used previously for experiments. Microscopic and cultural examinations of his stools were negative for amœboid organisms. He ingested cysts of *Entamœba histolytica*, strain *D*, mixed with powdered starch. Cultures of the stools of man 39 following the ingestion were negative. Microscopic examination of his stools showed *Entamœba histolytica* on the eleventh day after feeding and more or less constantly ever since. On the eighty-seventh day this man had a slight dysentery with abdominal pain and motile *Entamœba histolytica* in his bloody mucous stools, which lasted only one day and from which he recovered without treatment. He has been under observation one year since the beginning of the experiment, but has had no relapse of the dysentery.

These protocols are summarized in Table III.

As the protocols and Table III show, amœboid organisms could not be recovered in cultures on Musgrave and Clegg's medium from the stools of any of the men who had ingested *Entamœba histolytica*. On the other hand, *Entamœba histolytica* has been found microscopically in the stools of every man who became parasitized, and the entamœbæ have persisted in the stools of these men for an indefinite time. Therefore, it is demonstrated experimentally that *Entamœba histolytica*, like *Entamœba coli*, and in contrast to the *Amœbæ*, is an obligatory parasite which cannot be cultivated on Musgrave and Clegg's medium.

Of the 20 men who ingested *Entamœba histolytica*, 17 became parasitized at the first feeding, 1 required 3 successive feedings before becoming permanently parasitized, and 2, who did not become parasitized at the first feeding, were reserved as controls. Of the 16 men who ingested encysted *Entamœba histolytica*, 14, or 85.5 per cent, and of the 4 men who ingested motile *Entamœba histolytica*, 3, or 75 per cent, became parasitized. However, all of the men who ingested motile entamœbæ had the acidity of the contents of their stomachs neutralized with magnesium oxide. It is doubtful whether so large a percentage of them would become parasitized under natural con-

ditions. All 6 of the men who ingested encysted *Entamœba histolytica* without neutralizing the acidity of the gastric juices became parasitized.

The incubation period of the parasite, that is, the period of time elapsing between the ingestion of the infectious material and the appearance of the entamœbæ in the stools, is, as shown in these experimentally parasitized men, from one to forty-four days, with an average of nine days. In one case, man 44, in which microscopic examination of the stools was by an oversight not made between the sixth and thirty-third day, *Entamœba histolytica* was not found on the sixth and was found on the thirty-third day. If we exclude this case and that of man 5, who ingested motile entamœbæ and who had an abnormally long incubation period, forty-four days, the average incubation period of the parasite would be 5.7 days, which is approximately the same as in the case of *Entamœba coli*. It is probable that the number of entamœbæ ingested would in part account for the considerable variation in the incubation period in the different men.

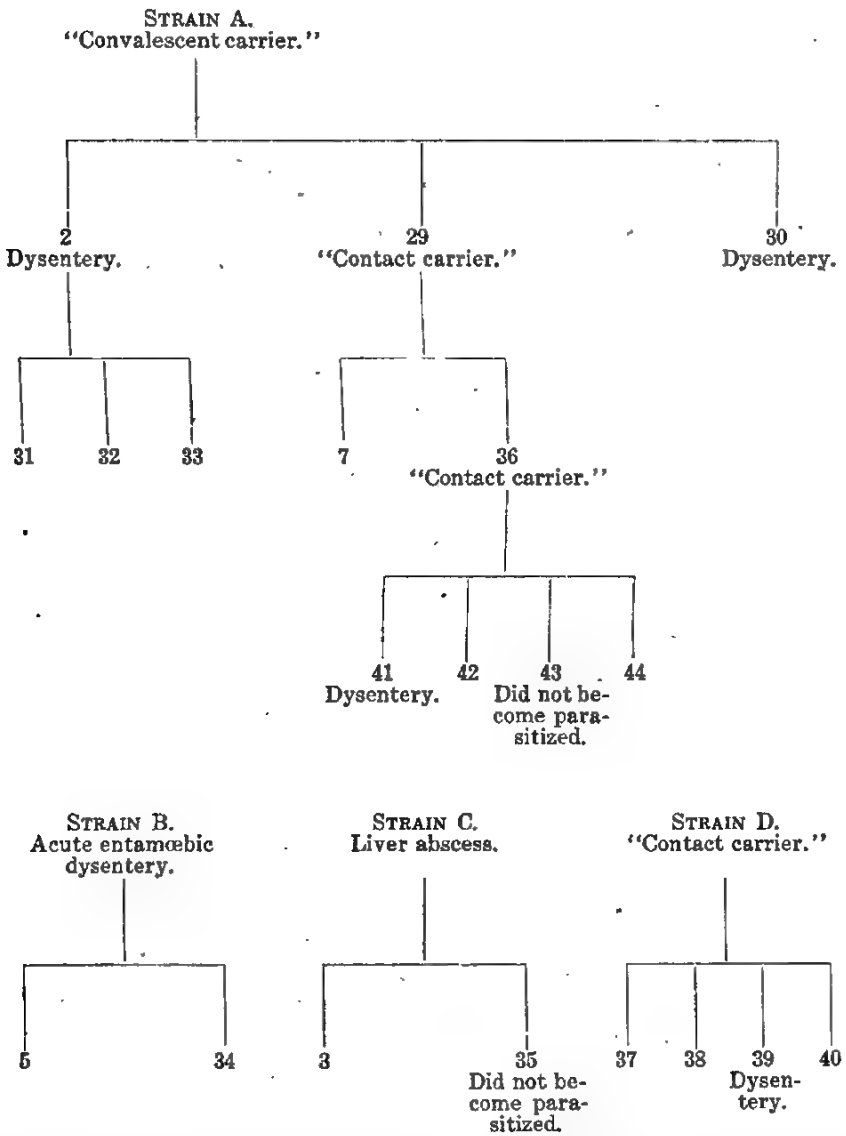
Of the 18 men experimentally parasitized with *Entamœba histolytica*, 4, or 22 per cent, have developed entamœbic dysentery. Two of these cases, men 30 and 32, had only slight attacks of dysentery, lasting one or two days, and recovered without treatment. The other 2 cases, men 2 and 41, had more severe attacks of dysentery lasting one or more weeks, and recovered only after receiving ipecac treatment. One of the latter, man 2, had a relapse thirty days after recovery which persisted until treated.

The 4 cases of experimental dysentery were obtained with material from 3 different sources and representing 2 distinct strains of *Entamœba histolytica*. Men 2 and 30 were infected with entamœbæ from the same individual, man *A*, who was convalescent eighty-four days from a slight attack of entamœbic dysentery of two days' duration. Man 41 ingested a strain of *Entamœba histolytica* coming originally from the same source as in cases 2 and 30, but which had been passed through two other men, 29 and 36, who did not develop and have not subsequently developed dysentery, before being ingested by man 42. Case 39 was obtained after the ingestion of a distinct strain of *Entamœba histolytica*, strain *D*, which came from a "contact carrier" of *Entamœba histolytica* without symptoms or previous history of dysentery.

The passages of the 4 strains of *Entamœba histolytica* in these

experiments and the cases of experimental dysentery are shown in the following diagram:

Diagram.



The incubation periods of these cases of experimental dysentery were twenty, ninety-five, eighty-seven, and fifty-seven days, respectively, with an average of 64.8 days. It is possible that the incubation period might in certain cases be shorter than

twenty days; on the other hand, on account of the latency characteristic of this disease, it is probable that the incubation period may often be much longer than ninety-five days.

The large percentage of latent infections (78 per cent) among these experimentally parasitized men was somewhat unexpected. However, the frequent occurrence of latent infections in entamœbic dysentery has been noted by a number of authors,[12] and is well known to every clinician and pathologist working in the Tropics.

Vincent (1909) has reported cases of entamœbic dysentery in soldiers returned from French Indo China, of whom some had never suffered from dysentery and others had recovered from attacks while in the Tropics. After their return to France, where infection was considered impossible, these men showed not the slightest intestinal symptoms for three, six, and, in 1 case, eleven months, and then developed attacks of entamœbic dysentery. In such cases Vincent observed that the attacks followed certain adjuvant causes, such as indigestion, alcoholic excess, chill, or excessive and prolonged fatigue.

Musgrave published in 1910 a study of 50 cases of intestinal entamœbiasis without diarrhœa which came to necropsy. Two of these cases showed a perforation of the appendix from entamœbic ulcerations, 5 had perforations of entamœbic ulcers of the large intestine, and 4 had entamœbic liver abscesses. The necropsies of the other 39 cases showed more or less extensive entamœbic ulcerations of the large intestine. None of these cases had bloody mucous stools or even diarrhœa; indeed, constipation was a characteristic feature of several of them up to the time of death.

Moreover, individuals who are infected with the pathogenic microörganism, but who show no clinical manifestations of the specific disease, are well known in most, if not all, infectious diseases. As an example of the prevalence of such "contact carriers," which is comparable with the condition found in entamœbic dysentery, the results of the investigation carried out under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York[13] may be cited. This investigation disclosed the fact that pneumococci could be isolated from the

[12] Dock (1891), Councilman and Lafleur (1892), Musgrave (1905), Martini (1908), Vincent (1909), Musgrave (1910).

[13] Park and Williams (1905), Longcope and Fox (1905), Duval and Lewis (1905), Buerger (1905).

mouths and throats of from 53 to 100 per cent of healthy persons, and that from 69 to 83 per cent of these organisms proved to be virulent on inoculating them into animals.

Therefore, it appears that the relatively small number of cases of dysentery obtained up to the present time in the men experimentally parasitized with *Entamœba histolytica* is thoroughly consistent with our knowledge of the prevalence of latent infections in this disease. It should also be borne in mind that not *Entamœba histolytica* alone, but all of the microörganisms contained in the dysenteric stool, were fed in each case and, consequently, the small percentage of dysenteries resulting from these feedings has no bearing upon the etiology, but is evidence only of the frequent latency, of this disease.

To what extent this latency, which is characteristic of entamœbic dysentery, is due to the chronicity of the ulcerative process, and whether or not the inability of *Entamœba histolytica* to penetrate the healthy intestinal epithelium plays a part in it, cannot at present be definitely answered. In the latter case the entamœbæ might be conceived to live as commensals in the intestine of their host, and only when there occurred some depression of the natural resistance of the host or of its tissues or some inflammation or actual lesions in the intestine, were the entamœbæ able to penetrate the intestinal epithelium, become tissue parasites, and produce the characteristic lesions of entamœbic dysentery.

It was hoped that information on this aspect of the subject might be obtained by post-mortem examination of men who had been parasitized for a considerable time with *Entamœba histolytica,* but who had never shown any dysenteric symptoms. However, only one necropsy has been obtained. This man, who had been parasitized with *Entamœba histolytica* for one hundred sixty-five days without showing any dysenteric symptoms, died of pulmonary and intestinal tuberculosis. Necropsy showed an extensive ulceration of the small intestine and cæcum which was clearly tubercular. In the lower large intestine there were a few small ulcers of doubtful anatomical character. Sections of these ulcers, however, showed no entamœbæ, but only large numbers of tubercle bacilli. The tubercular lesions in this case would seem to have afforded openings in the intestinal epithelium for the entrance of the entamœbæ, but the products of the tubercular ulcerations may have been inimical to the entamœbæ, and it is consequently unsafe to draw any conclusions from this case.

A further attempt was made to solve the question of latent infections by the experimental infection of animals, which could be killed at different times after infection and in which the behavior of the entamœbæ in the intestine could be studied post mortem. In these experiments 2 monkeys were fed repeatedly with fæces containing encysted *Entamœba histolytica;* 1 monkey was fed a dysenteric stool; 1 monkey received, *per œsophagus* after washing out the stomach with a suspension of magnesium oxide, a bloody mucous stool by stomach tube; 1 monkey received an injection of dysenteric stool into the rectum; 1 monkey received an injection of dysenteric stool into the cæcum; and 1 monkey received an inoculation of entamœbic liver-abscess pus into the liver. Two cats were fed encysted *Entamœba histolytica,* and 6 kittens received rectal injections of dysenteric stools containing motile *Entamœba histolytica.* One young pig was twice fed large numbers of encysted entamœbæ. All of the animals not only did not develop dysentery, but in no case have they become parasitized with *Entamœba histolytica.* These results are surprising in view of the experience of other authors who have found animals, especially cats and monkeys, readily infected with entamœbæ by feeding or rectal injections of dysenteric fæces. My results, while not numerous enough absolutely to exclude the possibility of parasitizing animals with *Entamœba histolytica,* at least indicate that they are less readily parasitized than men, 90 per cent of whom became parasitized in my experiments. In consequence of these negative results from attempts to infect animals with *Entamœba histolytica,* no further information was obtained in these experiments on the nature of latency in entamœbic dysentery.

Some light by analogy may be thrown on this subject by the results of experimental infections of monkeys with *Balantidium coli* which are now in progress. *Balantidium coli* produces a serious and often fatal dysentery in man, but latent infections which give rise to no clinical symptoms are as prevalent as in infections with *Entamœba histolytica.* In the experimental infections of monkeys, which almost invariably become parasitized after feeding encysted *Balantidium coli,* there is the same latency of clinical symptoms as in the human infections. One parasitized monkey killed one month after infection showed a slight colitis with the balantidia penetrating the sound tissues of the mucosa. Another monkey killed two months after infection showed no colitis or ulcerations. And a third monkey, which was dying five months after infection, was chloroformed, and at necropsy the large intestine was found to have an extensive

ulcerative colitis, although no dysentery had manifested itself during the five months the monkey had been infected. Therefore, so far as these experiments have gone, it appears that latent balantidiasis in the monkey may be due to several different causes, namely: (1) In certain cases to the failure of the balantidia to penetrate the tissues of the intestine, (2) in other cases to the chronicity of the ulcerative process, and (3) to the fact that more or less extensive colitis and ulceration may exist in the intestine of the infected animal without any dysenteric symptoms. It is conceivable, indeed probable, that the latency of entamœbic dysentery is due to similar causes.

Notwithstanding that the prevalence of latent infections has caused the results of the experimental infections with *Entamœba histolytica* to appear less clean-cut than the results with the *Amœbæ* and with *Entamœba coli* and has rendered the nonparasitized controls of these experiments inconclusive, nevertheless, I am of the opinion that these experiments supply evidence which, when considered with the morphological, clinical, and pathological data, is sufficient to establish beyond a reasonable doubt the etiologic relationship of *Entamœba histolytica* to endemic tropical dysentery.

In the first place, it is to be noted that the uniformly negative results of the feeding experiments with the *Amœbæ* and *Entamœba coli* suffice to exclude them once for all from the etiology of this disease. Therefore, the question of the etiologic factor must lie between *Entamœba histolytica* and some unidentified microörganism present in dysenteric stools.

If the entamœbæ are only commensals, the multiplication of which is favored by the dysenteric conditon and which invade the dysenteric lesions as a secondary infection, it would be expected that the common *Entamœba coli* would be most frequently associated with the disease; on the contrary, it is the relatively rare *Entamœba histolytica* only that is constantly found in the stools and lesions of entamœbic dysentery.

Post mortem, the entamœbæ are found not only in the lesions, but in the sound tissues underlying the lesions, where they are usually the only microörganisms demonstrable. They penetrate between the cells of the sound mucosa, the submucosa, and even of the muscularis, where by their migrations and multiplication, aided probably by the secretion of a ferment, they produce histolysis. They are found in the lymph spaces and in the blood vessels in which they are carried by the way of the portal circulation to the liver and give rise to abscesses as sequelæ to the intestinal infection. In these liver abscesses, which are usually

bacteriologically sterile, *Entamœba histolytica* is found not so much in the necrotic material as in the sound tissues at the borders of the abscess.

Anatomically and histologically both the intestinal and liver lesions are characteristic and do not correspond to lesions due to other microörganism. The typical ulcerations are of the so-called undetermined type, which undoubtedly result from the widespread wandering of *Entamœba histolytica* in, and the consequent histolysis of, the tissues underlying the intestinal epithelium. The cellular reaction about those lesions, when uncomplicated by secondary bacterial invasion, is not inflammatory but regenerative in character and consists not of polymorphonuclear leucocytes but of formative cells and lymphocytes. In liver abscesses, which are less frequently complicated by secondary bacterial infection than are the intestinal lesions, the nature of the morbid process is most clearly perceived. The so-called pus of these abscesses is not pus at all, but chiefly cellular detritus resulting from the histolysis of the liver tissue by *Entamœba histolytica.*

In the experimental infections with *Entamœba histolytica* no dysenteries have developed in the cases parasitized from a case of acute entamœbic dysentery, in those from an entamœbic liver abscess, nor in those who did not become parasitized with *Entamœba histolytica,* although the acidity of the stomach contents of all of these men was neutralized with magnesium oxide at the time of ingestion of the infectious material. If a bacterium or other unidentified microörganism should be concerned in the etiology of this disease, it would seem that these feeding experiments were made under conditions most favorable to secure infection.

On the other hand, all of the experimental dysenteries were obtained after ingesting *Entamœba histolytica* from healthy persons who were "carriers" of this parasite. Case 2 developed dysentery after ingesting *Entamœba histolytica* from a man convalescent fifty-nine days from a slight spontaneous attack of entamœbic dysentery, who has not subsequently shown any symptoms of dysentery, and who was, therefore, a "convalescent carrier" of *Entamœba histolytica.* Case 30 resulted from the ingestion of material from the same carrier convalescent one hundred sixty-one days. Case 39 suffered an attack of entamœbic dysentery after ingesting *Entamœba histolytica* obtained from a person who had not, and has not subsequently, developed

dysentery; that is, a "contact carrier." And case 41 developed entamœbic dysentery after ingesting *Entamœba histolytica* that had been passed through 2 "contact carriers." The history of this strain of *Entamœba histolytica* is as follows. It was originally from the same convalescent carrier (man A) from whom cases 2 and 30 were infected. *Entamœba histolytica,* from this "carrier" convalescent fifty-nine days, was ingested by man 29, who became parasitized, but did not develop dysentery. *Entamœba histolytica* from this "contact carrier," one hundred twenty-one days after infection, was next ingested by man 36, who likewise became parasitized, but did not develop dysentery. Finally, *Entamœba histolytica* from this second "contact carrier," one hundred thirty-four days after infection, was ingested by man 41, who became parasitized with *Entamœba histolytica* and developed entamœbic dysentery after fifty-seven days. Therefore, three hundred seventy-one days and the passage through 2 "contact carriers"—who had not, and have not subsequently, developed dysentery—separated this experimental case from the spontaneous case of entamœbic dysentery, a gap bridged by the innumerable and continuous generations of *Entamœba histolytica* in 4 different men.

No case of spontaneous entamœbic dysentery has occurred in this ward during the period of these experiments with *Entamœba histolytica.*

Therefore, it is believed that the results of these experiments warrant the conclusions that *Entamœba histolytica* is a strict or obligatory parasite, that it cannot be cultivated on Musgrave and Clegg's or other ordinary culture media, and that this entamœba is the essential etiologic factor in endemic tropical dysentery.

PART V. APPLICATION OF THE RESULTS TO THE DIAGNOSIS, TREATMENT, AND PROPHYLAXIS OF ENTAMŒBIC DYSENTERY

By Ernest Linwood Walker

The information concerning the etiology and endemiology of entamœbic dysentery and the morphology, biology, and parasitism of *Entamœba histolytica,* which has been obtained in the course of this experimental investigation, is believed to be of the greatest value for the diagnosis, treatment, and prophylaxis of this important tropical disease.

The identification of the specific microörganism in the laboratory constitutes the final word in diagnosis, upon which must

be based the treatment and prophylaxis of an infectious disease. On account of the relatively long incubation period usually existing in entamœbic dysentery, the prevalence of chronic and latent cases of the disease, and the frequent inefficience of treatment to kill all of the entamœbæ in the intestine, the microscopic identification of the pathogenic *Entamœba histolytica* is of particular importance in the diagnosis, the control of treatment, and the prophylaxis of entamœbic dysentery. Hitherto, on account of the uncertainty existing as to the specific entamœba concerned in the production of this disease and the supposed difficulty of identifying the organism, especially in the resting and encysted stage, the microscopic diagnosis of infections with *Entamœba histolytica* has been subject to many errors; indeed, in many laboratories, no attempt is made to distinguish between the pathogenic species and the common, harmless *Entamœba coli*, or to diagnose latent and chronic infections. An extensive practical experience gained in the course of this investigation has demonstrated that the microscopic diagnosis of infections with *Entamœba histolytica* can be made with certainty, and has disclosed many practical points in the technique and the application of this diagnosis. Therefore, it is believed that a somewhat extended treatment of this subject is warranted.

The material for the microscopic examination for *Entamœba histolytica* should be a stool obtained, contrary to the prevailing practice, without the previous administration of a purgative. In stools obtained after a purgative, *Entamœba histolytica*, if present in the fluid stool, is in a preëncysted stage at which it most closely resembles the nonpathogenic species, *Entamœba coli;* consequently, a differential diagnosis between the two species is difficult and often impossible.

It may be objected that without a purgative infections with *Entamœba histolytica* will frequently be overlooked. However, such is not the case. It has been my experience in following many cases of entamœbic infection with daily stool examinations, including cases doubly infected with *Entamœba histolytica* and *Entamœba coli*, that the entamœbæ are rarely absent from the normal stools several successive days and that *Entamœba histolytica* is more constantly present, and usually present in larger numbers, in the stools of infected persons than is *Entamœba coli*. In 930 microscopic examinations made of stools, without the previous administration of a purgative, from men known to be parasitized with *Entamœba histolytica*, and who were not undergoing treatment, the entamœbæ were found 664 times, or 71.39 per cent; that is, in nearly 3 out of every 4 of such exam-

inations. Moreover, the negative results were based on the examination of a single coverslip which was often hurriedly made. The examination under similar conditions of 303 stools of men known to be parasitized with *Entamœba coli* showed the entamœbæ in 171, or 56.44 per cent of the examinations; in other words, in about 1 out of every 2 of such examinations.

A further objection, that may be raised to the examination of formed stools, is the fact that in such stools usually only encysted entamœbæ are to be found. It is an opinion generally held, and which is supported by the statement in all textbooks, that a positive diagnosis of entamœbic infection should never be made unless motile entamœbæ are observed. It is of the greatest importance, however, for the diagnosis of chronic and latent infections that one should be able to distinguish resting and encysted entamœbæ from other bodies found in fæces and to differentiate the cysts of *Entamœba histolytica* from those of *Entamœba coli*. This can be done with certainty by the experienced protozoölogist. The majority of the 1,233 examinations mentioned in the preceding paragraph were made of formed stools containing nonmotile and encysted, chiefly encysted, entamœbæ. Moreover, it is the encysted stage of the entamœba that furnishes the most unequivocal characters for the differentiation of the pathogenic *Entamœba histolytica* from the harmless *Entamœba coli*.

In the examination of liver-abscess pus for *Entamœba histolytica*, the pus first obtained after the operation usually does not contain entamœbæ; frequently they appear in the pus from the drainage tube only after several days. The explanation of this is to be found in the fact that the entamœbæ are not found in the pus of the abscess, but only in the tissues at the borders of the abscess. Consequently, it is only when the borders of the abscess begin to slough off that the entamœbæ appear in the drainage from the abscess. Therefore, a negative diagnosis of entamœbic liver abscess should never be made except after negative examinations obtained for several successive days after operation.

Dysenteric or diarrhœal stools should be examined as soon as possible after they are passed, since the motile entamœbæ present in such stools quickly die and disintegrate. On the other hand, in the formed stools of chronic and latent infections, the encysted entamœbæ persist unchanged for days, and consequently the examination can be made at one's leisure.

In making the examination, a small platinum loopful of the fluid or semifluid material should be placed on a microscope

slide and the cover-glass dropped upon it. Slight pressure may be exerted, if necessary, upon the cover-glass with the forceps to cause the material to flow as a thin film between the cover-glass and slide. If the stools be more or less formed, a small drop of water should be placed upon the slide and a minute portion of the stool rubbed up in it, forming a fairly thick suspension of the fæces in the water, upon which the cover-glass should be placed. A satisfactory preparation must be thin, but there should not be an excess of fluid which will permit the cover-glass to float about when the oil-immersion objective is applied to it. A warm stage is not necessary for making the examination.

The advantage of a preliminary examination of the preparation with low magnification (Leitz 3 or Zeiss AA objective) cannot be overestimated. It enables the examiner to make a rapid survey of the whole preparation and to pick out the individual entamœbæ for examination with the oil-immersion objective. When the entamœbæ are few in the preparation, they can be found with difficulty, if at all, with higher magnification. With a Leitz 3 or Zeiss AA objective and a 3 ocular, the entamœbæ appear as round, oval, or irregular, colorless, and refractive dots which with proper focusing stand out distinctly in the background of the preparation. Practical experience will enable the microscopist to distinguish them from certain other bodies that are met with in stools. By applying the oil-immersion objective—most conveniently used with the dry objective on a revolving nose-piece—to every body in the preparation which looks suspicious under low magnification, this experience will soon be attained. Indeed, it is not only possible for the experienced microscopist to identify an entamœba with the low magnification, but to distinguish a cyst of *Entamœba histolytica* from one of *Entamœba coli* with a considerable degree of certainty by the difference in its size and refractiveness.

A suspected entamœba, having been located in the preparation with the low-power objective, should then be examined with the 1/12 oil-immersion objective. With this magnification the entamœbæ present certain morphological characters that enable the experienced investigator to identify them whether they be in the motile, resting, or encysted stage.

The motile forms will give little difficulty, even to the novice, since their movements are characteristic.

The resting entamœba is distinguished from other bodies found in the stool by its size, distinctness, regularity of contour, degree of refractiveness, and especially by its nuclear structure.

The entamœbæ vary in size within considerable limits, but are usually from 20 to 30 microns in diameter. They are, therefore, larger than pus cells, or other protozoa, with the exception of *Balantidium coli*, that are found in the stools of man. They are also more refractive than pus, epithelial, or other cells found in the stools. The nuclear structure of the entamœba is particularly characteristic. The unencysted entamœba possesses, unless in the process of division, only a single nucleus. This nucleus is round, or occasionally slightly oval or irregular, small with reference to the size of the cell, and appears not solid but as a refractive ring (Plate I, figs. 3, 5, 6, 7). This relatively small, ring-shaped nucleus appears to be absolutely diagnostic of an entamœba. Only one other kind of cell observed in stools possesses a nucleus in any way resembling that of an entamœba. This is an epitheloid cell, sometimes found in mucous stools, which has a ring-form nucleus relatively much larger than that of an entamœba, occupying one-fourth to one-half of the cell. While an entamœba may occasionally be observed with an abnormally large nucleus, probably preparatory to division, the nucleus never approaches the size of the nucleus of this epitheloid cell. The latter cells are also less refractive and granular than entamœbæ.

The encysted entamœba is round or slightly oval, more refractive than the resting or motile stage, and is surrounded by a more or less distinct cyst wall. The nuclear structure here also is characteristic. The cyst contains several (from 2 to 8, depending upon the species of entamœba and the stage of development of the cyst) ring-form nuclei usually smaller than, but of the same structure as, the nucleus of the motile entamœba (Plate I, figs. 4 and 8).

The technique and descriptions so far given refer to the examination of living entamœbæ in fresh stools. This method of stool examination for entamœbæ is the quickest and for general purposes of diagnosis the most satisfactory. The preparation of stained specimens takes more time and a more extensive technique, and certain distinctive characters of the entamœba are lost in the fixed and stained preparation. On the other hand, staining sometimes assists in bringing out the details of nuclear structure, and is necessary for making permanent preparations of entamœbæ.

The technique of fixing and staining entamœbæ which has given the most uniformly satisfactory results is as follows. A thin smear of the fresh fæces or liver-abscess pus is made on a cover-glass, fixed in sublimate-alcohol mixture or Zenker's

fluid for from five to fifteen minutes, thoroughly washed in distilled water, stained in aqueous alum hæmatoxylin from three to five minutes, washed in distilled water, passed through successive grades to absolute alcohol, cleared in xylol or oil origanum, and mounted in xylol-balsam. All of the stages of this process are most conveniently carried out by floating the cover-glass, preparation downward, upon the surface of the different liquids contained in watch glasses. The preparations should be fixed moist and should not be allowed to become dry throughout the process of staining and mounting.

The sublimate-alcohol mixture consists of 2 parts of a saturated aqueous solution of mercuric chloride and 1 part of absolute alcohol. The sublimate solution should be saturated warm and should be kept in stock. The absolute alcohol should be added in proper proportion only at the time of using, because alcohol evaporates and the solution changes in standing.

The aqueous alum hæmatoxylin has the following composition:

Hæmatoxylin crystals	1
Saturated aqueous solution of ammonia alum	100
Distilled water	300
Thymol	a crystal.

The hæmatoxylin crystals are dissolved in the water by the aid of heat, and the other substances added to the solution. The stain should be ripened for from a week to ten days in a flask or bottle loosely plugged with cotton. It is then ready for use and should be kept in a tightly stoppered bottle away from the light. It will keep in good condition for several months.

Bodies that are liable to be mistaken for entamœbæ in the stools include air bubbles, fat globules, starch or proteid grains, pus and epithelial cells of the host, and certain unicellular vegetable organisms. Of these air bubbles, fat globules, and starch or proteid granules of undigested food, while possibly deceptive with low magnification, should from their structure cause no difficulty when examined with high magnification. Stools containing mucus or pus often contain many cells which are confusing to the inexperienced microscopist. It will assist the observer if he remembers that these pus and epithelial cells with few exceptions are distinctly smaller than entamœbæ. It will, therefore, be necessary only to take into consideration cells which, when viewed with the low magnification, are distinctly larger than the average.

In fæces containing pus there are sometimes present large round cells of uncertain identity which in size and general appearance

closely resemble resting or encysted entamœbæ. The cells contain from one to several small, round or irregular, refractive, nucleus-like bodies that stain like chromatin. It is possible that they are cells showing degenerative changes with fragmentation of the nucleus. These cells are, however, readily distinguished with high magnification from entamœbæ by the structure of the nucleus-like bodies, which are not ring-form, but solid chromatin masses. Motile forms of entamœba also will be frequently found in such stools, which will aid in the diagnosis.

Certain unicellular vegetable organisms known as *Blastocytis hominis* Brumpt, which are believed to be allied to the yeasts, are found rather frequently in the stools of man in the Tropics. Smaller forms of these cells have been mistaken for the cysts of *Trichomonas intestinalis*, and the larger forms simulate the encysted entamœbæ very closely in size and general appearance. They are, however, slightly less refractive than the cysts of *Entamœba*, and can, therefore, be distinguished by the experienced observer, even with low magnification. Under high magnification they are seen to have a wholly different structure from the cysts of *Entamœba*. They are round, oval, or slightly irregular, and possess a distinct wall. The protoplasm is restricted to several narrow segments of the cell, and contains from one to several granules staining like chromatin. The main body of the cell is filled with a homogeneous, hyaline, slightly refractive, and often faintly yellow mass, the nature of which is doubtful, but it probably represents reserve food substance.

An examination of figs. 3 to 8 on Plate I will give a good idea of the general morphology of the entamœbæ. Figs. 1 and 2 show the motile encysted stages of a typical nonparasitic amœba for comparison with the entamœbæ.

The differentiation of *Entamœba histolytica* from *Entamœba coli* depends upon certain morphological characters of the two species which are very distinctive at certain stages, but less distinctive at other stages, of the development of the two species. These stages of the development of the parasites are correlated with the clinical manifestations of the infection and especially with the consistence of the stools of the host. Therefore, the comparative morphology of *Entamœba histolytica* and *Entamœba coli* are most conveniently discussed in relation to the nature of the stools in which they are found; namely, (1) in dysenteric stools, (2) in diarrhœal stools and stools after a purgative, and (3) in formed stools.

1. IN DYSENTERIC STOOLS

Both *Entamœba histolytica* and *Entamœba coli* occur only in the motile stage in dysenteric stools; and, when double parasitization exists, *Entamœba histolytica* is usually the more numerous in such stools.

Size.—The size of both *histolytica* and *coli* are subject to wide variations, and little dependence can be placed on this character for diagnostic purposes. In dysenteric stools *histolytica* often appears larger than *coli* (Plate I, figs. 3 and 5). That this larger size of *histolytica* is only apparent, and not real, is probable from the fact that in the encysted stage (the only stage in which reliable measurements can be made) *histolytica* is almost invariably smaller than *coli* (Plate I, figs. 4 and 8). This apparently larger size of motile *histolytica* is probably connected with the more active movements of this species; while *coli* is sluggishly motile and tends to retain a more or less spherical shape, *histolytica* is actively motile and is extended flat over the surface of the substratum.

Shape.—Entamœba histolytica, being more actively motile than *Entamœba coli,* presents a more varied form to the observer. While *coli* is usually round, oval, or slightly irregular, *histolytica* is more often long oval, ligulate, or irregular in fresh dysenteric stools.

Appearance.—Entamœba histolytica is hyaline and feebly refractive while *Entamœba coli* is more porcelaneous and refractive in appearance.

Motility.—The amœboid movements of *Entamœba histolytica* are very active in fresh dysenteric stools, and the motility of this species often persists for some hours after the stool has become cold. On the other hand, the movements of *Entamœba coli* are always sluggish, and all motility is usually soon lost in cold stools.

Cytoplasm.—The cytoplasm of *Entamœba histolytica* is homogeneous, and in the stained entamœba is seen to have a coarsely reticulated structure (Plate I, fig. 5). In cold stools it frequently appears much vacuolated. Contrary to the description given by some authors, there is no true distinction to be seen between ectoplasm and entoplasm in the resting entamœba. Individual entamœbæ, which contain granular material from partly digested food, sometimes present the appearance of a granular entoplasm. In motile *histolytica* the extended pseudopods may present a more dense, hyaline appearance than the reticulated body of the cytoplasm. The cytoplasm of *histolytica* may contain

various cells, including red blood corpuscles, of its host. On the other hand, the cytoplasm of *Entamœba coli* is more granular in appearance (Plate I, fig. 3). A hyaline ectoplasm is apparent only in the pseudopods of the motile entamœba. The cytoplasm of *coli* more often contains bacteria, starch and proteid grains, and other débris from the fæces than cells from the body of its host. The absence from the cytoplasm of red blood corpuscles and other cells of its host may result rather from the fact that *coli* is a strict commensal and is more often found in non-dysenteric stools than from its incapacity to ingest red blood corpuscles.

Nucleus.—The nucleus of *Entamœba histolytica* is usually indistinct in the motile organism, especially if it be actively motile or much vacuolated. In the latter case it is sometimes impossible to distinguish the nucleus from the vacuoles. In stained preparation the nucleus of *histolytica* is seen to possess a thin membrane and to be relatively poor in chromatin. This chromatin is distributed as a thin peripheral layer or as scattered granules about the inner surface of the nuclear membrane, with a few granules scattered in the network of the nonrefractive, nonstainable nucleoplasm (Plate I, fig. 5). This type of nucleus is characteristic of *histolytica* found in stools in acute dysentery that consist exclusively of mucus and blood. In stools of chronic cases of dysentery that consist of more or less mucus and blood mixed with fæcal matter, the so-called *"tetragena"* type of nucleus is commonly met with. This type of nuclear structure contains more chromatin than the preceding type, and the chromatin has a characteristic arrangement. It is distributed partly as a more or less extensive but loose layer, which frequently shows radial projections, about the inner surface of the nuclear membrane, and partly as a loose central karyosome of varying structure. This karyosome consists typically of a central granule, the "centriol," surrounded by a clear halo that is bounded by a layer of chromatin granules (Plate I, fig. 6). All intermediate stages between the typical *histolytica* and the *"tetragena"* types of nuclei are to be observed in dysenteric stools. On the other hand, the nucleus of *Entamœba coli* is distinctly visible in the living and motile entamœba as a heavy refractive ring. It consists of a nuclear membrane and a relatively large amount of chromatin which is arranged as a heavy, dense, continuous or broken layer about the inner surface of the nuclear membrane and sometimes also in a small, central, dense karyosome. The interior of the nucleus consists of a nonrefractive, nonstainable

nucleoplasm (Plate I, fig. 3). Therefore, the nucleus of *Entamœba histolytica* differs from that of *Entamœba coli* in being less distinct, often invisible, in the living entamœba, and in being poorer in chromatin. The *"tetragena"* type of *Entamœba histolytica* found in stools of chronic cases of dysentery has a nucleus more closely resembling that of *Entamœba coli;* but the peripheral layer of chromatin is less dense, often shows radial projections, and the karyosome is loose instead of dense in structure.

2. IN DIARRHŒAL STOOLS

In diarrhœal stools and stools after a purgative, *Entamœba histolytica* is usually small, sluggishly motile or immobile, and possesses a nucleus that is distinctly visible in the living entamœba as a more or less heavy peripheral ring of chromatin (Plate I, fig. 7). Therefore, it more or less closely resembles *Entamœba coli.* These forms appear to represent changes in *Entamœba histolytica* preparatory to encystment. They are spoken of by Darling (1913) as the "small generation" of *Entamœba histolytica,* and were mistaken by Elmassian (1909) for a distinct species of *Entamœba.* Although the small size in part may be due to less volume, it is probable that it results in part from the contraction and rounding up of the much extended motile entamœba. The increase of chromatin content of the nucleus may be considered as a preparation for the multiple nuclear division that is to take place in the cyst. While all stages from the typical *histolytica* through the *"tetragena"* to this preencysted stage of *Entamœba histolytica* may be found in diarrhœal stools or stools after a purgative, the predominance of the preëncysted stage and the more or less resemblance of it to *Entamœba coli* make the differentiation of the two species difficult and sometimes impossible in such stools.

3. IN FORMED STOOLS

In formed stools both *Entamœba histolytica* and *Entamœba coli* are present in the encysted stage, and it is this stage of the entamœba that presents the most distinctive character for making a differential diagnosis. Furthermore, the identification of *Entamœba histolytica* in the encysted stage in formed stools is extremely important for the diagnosis of chronic and latent infections and for the control of treatment of entamœbic dysentery, and constitutes one of the most efficient factors in the prophylaxis of this disease.

The cysts of *Entamœba histolytica* (Plate I, fig. 8) are relatively small, from 10 to 15 microns in diameter. They are round, or occasionally oval, moderately refractive, and have a thin cyst wall. The completely encysted entamœba contains 4 ring-form nuclei and, usually, one or more elongated refractive bodies, that stain with chromatin stains and which have been designated by Hartmann as chromidial bodies. On the other hand, the cysts of *Entamœba coli* (Plate I, fig. 4) are larger (from 15 to 20 microns in diameter), more refractive, and usually possess a thicker cyst wall. The completely encysted entamœba contains 8 (occasionally more) nuclei and does not include "chromidial bodies." The encystment of *Entamœba coli* appears to proceed more rapidly than that of *Entamœba histolytica*, so that from 2 to 6 nuclear stages are infrequently met with. In the case of *Entamœba histolytica*, nuclear multiplication appears to take place early, so that from 2 to 4 nuclear stages are frequently seen before encystment is complete; indeed, occasionally in the motile entamœba.

For convenience of reference, the more distinctive and constant characters of *Entamœba histolytica* and *Entamœba coli* are tabulated.

Motile stage.

A. *Entamœba histolytica.*	B. *Entamœba coli.*
1. Appearance hyaline.	1. Appearance porcelaneous.
2. Refractiveness more feeble.	2. Refractiveness more pronounced.
3. Movements active in the fresh stools.	3. Movements sluggish.
4. Nucleus more or less indistinct.	4. Nucleus distinct.
5. Chromatin of nucleus scanty.	5. Chromatin of nucleus abundant.

Encysted stage.

A. *Entamœba histolytica.*	B. *Entamœba coli.*
1. Cyst smaller.	1. Cyst larger.
2. Cyst less refractive.	2. Cyst more refractive.
3. Cyst usually contains elongated refractive bodies known as "chromidial bodies."	3. Cysts do not contain "chromidial bodies."
4. Nuclei never more than 4.	4. Nuclei 8, occasionally more.
5. Cyst wall thinner.	5. Cyst wall thicker.

Therefore, in dysenteric stools and sometimes in diarrhœal stools, the characters of the motile *Entamœba histolytica* are fairly distinctive, and the experienced observer will have little difficulty in identifying the species. Unusually, however, in diarrhœal stools and in stools after a purgative *Entamœba histolytica* is in a preëncysted stage in which it closely resembles *Entamœba coli*, especially in its sluggish motility and its distinct

nucleus containing much chromatin. It is for this reason that I have insisted upon stool examinations without the administration of a purgative. In the case of natural diarrhœal stools, diagnosis can usually be made by an experienced protozoölogist by a careful study of the stools on successive days; but it is always advisable to endeavor to obtain a formed stool. Formed stools, when they can be obtained, are always to be preferred for making a laboratory diagnosis of entamœbic infection, because the encysted entamœbæ in such stools present the most distinctive morphological characters for the differential diagnosis between *Entamœba histolytica* and *Entamœba coli.* Finally, it is to be insisted upon that a negative diagnosis should never be made on a single stool examination, since the entamœbæ may occasionally be absent from the stools of an infected person; nor upon the identification of *Entamœba coli* in a stool, since there may exist a double parasitization with this species and *Entamœba histolytica.* In all such cases a diagnosis should be based on several examinations made on different days.

The treatment of entamœbic dysentery in the Philippines has been based hitherto upon the presence of entamœbæ in the stools without regard to the species. With the establishment of a morphological and pathogenic distinction between *Entamœba histolytica* and *Entamœba coli,* and the consequent ability to make a differential diagnosis between the two species, there no longer exists a justification for the indiscriminate treatment of every person showing entamœbæ in his stool. *Entamœba coli* is a very common commensal of man in the Tropics, but it is usually present in small numbers in the intestine and is harmless. Consequently, there is no reason why a patient parasitized with this species should, unless he desired, be subjected to the more or less disagreeable course of treatment. The indiscriminate treatment of all persons showing entamœbæ in their stools is as indefensible as would be the treatment with diphtheria antitoxin of every person showing a culture of any bacillus whatsoever from his throat.

The evidence so far secured in this investigation points to the conclusion that the ordinary routine treatment with ipecac, while efficient in relieving attacks of dysentery and in causing the entamœbæ to disappear temporarily from the stools, frequently does not kill all of the entamœbæ in the intestine; consequently, the patient is liable to a relapse of the dysentery. This tendency to relapse after chemotherapeutic or drug treatment is, as is well known, characteristic of other protozoan and of spirochæte infections. Two acute attacks and 1 relapse of dysentery and 4

latent infections with *Entamœba histolytica* have been treated during this experimental investigation and followed with microscopic examinations of the stools. While the dysenteric symptoms, in such cases as they existed, were always promptly relieved and the entamœbæ in both the acute and the latent cases always disappeared temporarily, the entamœbæ in every case, except one, reappeared in the stools of the patient in from ten to fifteen days after treatment. In one case of latent infection the entamœbæ disappeared from the stools of the patient after treatment and were absent for thirty days, when he was discharged from the hospital and passed from observation. A further study of the efficience of ipecac and of the soluble salts of emetine in killing all of the entamœbæ in the intestine of the patient, especially of latent cases, is greatly to be desired. The effects of varied doses, the administration by different methods, and especially the tests of prolonged and repeated treatment, controlled by daily stool examinations over long periods of time, should be investigated. Ipecac, especially its alkaloid emetine, is probably the most efficient drug that we possess for the treatment of entamœbic dysentery, but it is extremely important that a method of treatment be worked out that will permanently free the intestine of the patient from entamœbæ in order to prevent relapses and to repress "carriers."

In consequence of the frequent failure of ipecac treatment as at present administered to kill all of the entamœbæ in the intestine of infected persons, treatment should always be controlled by stool examinations. The usual routine examinations made during and immediately after treatment are useless, since the entamœbæ almost always disappear temporarily after treatment. The examinations should be made at frequent intervals for some months after treatment, and if the entamœbæ reappear in the stools the treatment should be repeated. With this precaution it is believed that relapses, so common in entamœbic dysentery, can be prevented.

The prophylaxis of entamœbic dysentery in many, if not most, parts of the Tropics has been based upon the erroneous conceptions concerning the etiologic agent of this disease. In consequence of the cultivation and infection experiments of Kartulis (1891), Celli and Fiocca (1894), Musgrave and Clegg (1904), Noc (1909), Greig and Wells (1911), Gauducheau (1912), Chatton and Lalung-Bonnaire (1912), and others, together with gross carelessness of investigators in the identification of species of amœboid organisms, the opinion has been widely held, at least in the Far East, that, if not all amœbæ living

in water and other external sources are capable of living parasitically in the intestine of man and of producing dysentery, at least the pathogenic species is capable of living and multiplying indefinitely outside of the body of its host. Such a characteristic of *Entamœba histolytica* would be unique among pathogenic microörganisms, and would, indeed, constitute entamœbic dysentery the most formidable disease of mankind and the least amenable to prophylaxis. Not only the water, but everything in the Tropics, even the air, contains amœbæ, motile or encysted in greater or lesser numbers, and efficient preventive measures against this disease would be practically impossible.

On the other hand, the experimental determination that entamœbic dysentery is caused by one species of amœboid organism only, and that this species is a strict or obligatory parasite which cannot multiply outside of the body of its host, profoundly limits the prophylactic problem of this disease; indeed, reduces it to almost, but not quite, the same level as that of other intestinal infectious diseases, such as bacillary dysentery, typhoid fever, and cholera. Every case of entamœbic dysentery, under these conditions, must arise directly or indirectly from some preceding case of entamœbic dysentery, and the prophylactic problem becomes that of protecting the well from cases of the disease, the sanitary disposal of the dejecta of the diseased, and the detection and treatment of "carriers" of the pathogenic entamœba.

Every acute case of entamœbic dysentery is constantly passing in his stools greater or smaller numbers of *Entamœba histolytica*; but in dysenteric stools the entamœbæ are all in the motile stage, in which they are probably less resistant to external influence than any other intestinal organisms. They not only will not live, but even disintegrate within a few hours after being passed in the fæces. It is also probable that in this stage they are incapable of surviving passage through the normal stomach, but are destroyed by the acidity of its contents. Of the 4 men who ingested the motile *Entamœba histolytica* in my experiments, 3, or 75 per cent, became infected; but these infections were secured under the most favorable circumstances, large numbers of the organism being ingested, together with magnesium oxide to neutralize the acidity of the stomach. It is unfortunate that some of these men were not fed the entamœbæ without neutralizing the acidity of the stomach contents, in order to determine experimentally the possibility of infecting with the motile stage under natural conditions. Darling (1913) states that infections invariably fail when only the motile (trophozoite) stage of *Entamœba histolytica* is fed to kittens. Shirota (1912) makes a

similar statement as a result of his experience. The purpose of my experiments as performed was to obtain parasitization and to secure the most favorable conditions possible for infection with any other organisms that, associated with the entamœba, might be an etiologic factor in producing dysentery. In consequence of the extremely feeble resistance of the motile *Entamœba histolytica* to external influences, it is not considered that cases of acute entamœbic dysentery are an important source of infection.

On the other hand, it is believed that chronic and latent cases of this disease are the chief, if not the exclusive, source of infection in endemic regions, first, because of their relative prevalence; secondly, because this condition persists indefinitely; thirdly, because their infection is unsuspected; and, fourthly, because these "carriers" are constantly passing in their stools, often in enormous numbers, the resistant, encysted stage of *Entamœba histolytica.*

From the results of my experimental infections it appears that 78 per cent of persons parasitized with *Entamœba histolytica* become "contact carriers" of the parasite. For every case of dysentery obtained in these experiments, there were 5 cases of latent infection; and of the 4 cases of dysentery, 2 cases were chronic, and the 2 acute cases became "convalescent carriers" of *Entamœba histolytica.* In the examination of 101 healthy men in Bilibid Prison, who had not been used for experiments, 9, or 8.9 per cent, were found to be "carriers" of *Entamœba histolytica.* These men had all been in the prison for years, and it is consequently probable that the percentage of "carriers" was lower than would be found outside.

While acute entamœbic dysentery lasts only days or weeks, the chronic and latent infections persist indefinitely. None of the 20 experimentally infected nor the 9 naturally infected men has ceased to be a "carrier" of *Entamœba histolytica,* although some of them have been under observation for over two years. The longest time of which I have an accurate record of a man carrying *Entamœba histolytica* is two years and four months, and it still appears in his stools in undiminished numbers. Moreover, as we have seen, the ordinary routine treatment of such "carriers" may not permanently remove the parasites from the intestine.

These "carriers" are constantly passing in their stools the resistant, encysted stage of the pathogenic entamœba. As has been stated previously in another connection, in 930 stool examinations of men known to be parasitized with *Entamœba histo-*

lytica, the entamœbæ were found 664 times, or in 71.39 per cent of these examinations. The majority of these examinations were of "carriers" who showed no dysenteric symptoms, but who were passing the encysted entamœbæ, often in enormous numbers, in their formed stools. These encysted entamœbæ, while incapable of multiplication or other vital activities outside of the body of their host, are resistant to external influences and are consequently capable of maintaining their vitality for some time outside of the body and of passing uninjured through the stomach of their host. Observations on the resistance of the encysted stage of *Entamœba histolytica* have not been as numerous in these experiments as could be desired. However, experiments were made with the cysts of *Entamœba histolytica* kept two days and with cysts of *Entamœba coli* kept ten days at tropical temperature. In both cases the cysts were kept moist. Parasitization was obtained in every case with this material. Darling (1913) put fæces containing cysts of *Entamœba histolytica* in 10 volumes of sterile tap water for three days. He was unable to infect 2 kittens or to find any cysts of the entamœbæ after this treatment, and concluded that the cysts disappear when in contact with water for this length of time. I have no data on the effect of drying upon the vitality of the cysts. Schaudinn (1903), however, infected kittens with fæces containing *Entamœba histolytica* air-dried for six weeks. On the other hand, Darling (1913) failed to infect 2 kittens with fæces containing cysts of this entamœba that had been dried in air for seven weeks. With regard to the resistance of the cysts of entamœbæ to the gastric juices in passage through the stomach, the following data were secured in these experiments. Of 12 men who ingested encysted *Entamœba coli* without neutralizing the acidity of the contents of their stomachs, 11 became parasitized; and of 6 men who ingested encysted *Entamœba histolytica* under similar conditions, all become parasitized.

The knowledge of the part which these "carriers" of *Entamœba histolytica* probably play in the spread of entamœbic dysentery, together with the ease and certainty with which such "carriers" can be detected by microscopic examination of their stools, makes the prophylaxis of this disease relatively simple. It is believed that it would be possible, were it practicable, to eradicate this disease from any region by a systematic examination of stools and the treatment or isolation of all persons found to be carriers of *Entamœba histolytica*. In the absence of such thoroughgoing prophylactic measures, a sanitary disposal of all fæcal matter should be insisted upon and household "carriers" of *Entamœba*

histolytica should be eliminated. Native household servants who cook and handle food, who are usually more or less uncleanly in their habits, and some of whom are carriers of *Entamœba histolytica*, are believed to be one of the chief sources of infection of white persons residing in the Tropics; and, as a most essential prophylactic measure, stool examinations should be made of all such servants, and those found infected should be discharged or subjected to treatment.

Equally important is the matter of personal prophylaxis. On account of the relatively long incubation period of the disease and the frequent occurrence of chronic and latent infections, it will usually be possible to anticipate with treatment an attack of dysentery. If persons residing in endemic regions should have frequent stool examinations made by a competent protozoölogist and, if at any time parasitization with *Entamœba histolytica* be discovered, should undergo treatment, it is believed that it would rarely be necessary for a person to suffer from entamœbic dysentery. A stool examination made once a month would ordinarily be sufficient to anticipate an attack of dysentery. Such a procedure would constitute a most efficient method of personal prophylaxis.

PART VI. SUMMARY AND CONCLUSIONS

By Ernest Linwood Walker

This investigation was undertaken to determine experimentally the etiologic relationship of different species of amœboid organisms to endemic tropical dysentery. It has consisted of 60 feeding experiments with the different species of *Amœba* and *Entamœba* that have been implicated in the production of this disease.

These experiments differ from those hitherto performed (1) in the number of comparative tests made of different species; (2) in that the experiments have been more carefully controlled and especially in that the species of amœboid organism fed to, and recovered from, the experimental animal in every case have been determined; and (3) in the fact that the experiments have been made not upon the lower animals but upon man.

A. Twenty feedings of cultures, representing 13 strains and 8 species of *Amœba*, isolated from the Manila water supply and other nonparasitic sources, from the stools of healthy persons or persons suffering from diseases other than dysentery, and from dysenteric stools, have been given to 10 different men, with the following results:

1. The *Amœbæ*, when ingested by men, can usually be recovered in cultures from their stools on Musgrave and Clegg's medium during the first few days after feeding, but never subsequently.

2. Microscopic examination of the stools of men after ingesting cultures of amœbæ have been invariably and constantly negative.
3. None of the men who ingested cultures of amœbæ have developed dysentery.
4. Therefore, the following conclusions appear to be warranted:
   a. The cultivable amœbæ are incapable of living parasitically in the intestinal tract of man.
   b. The amœbæ, when obtained in cultures from stools, intestinal contents, or liver-abscess pus, are derived either from cultural contaminations or from encysted amœbæ which have been ingested with water or food and have passed unchanged through the intestinal tract.
   c. The cultivable amœbæ are nonpathogenic, and consequently play no rôle in the etiology of endemic tropical dysentery.

B. Twenty feedings with 5 strains of *Entamœba coli* have been given to 20 different men with the following results:
1. Cultures on Musgrave and Clegg's medium of the stools of men who have ingested *Entamœba coli* have been invariably negative.
2. On the other hand, *Entamœba coli* has been found microscopically, after a short incubation period, in the stools of every man who became parasitized, and the entamœbæ have persisted in the stools of these men for an indefinite time.
3. Of the 20 men who ingested *Entamœba coli*, 17 became parasitized at the first feeding and 3 who did not become parasitized were reserved as controls.
4. The incubation period of *Entamœba coli*, as determined by these experimental parasitizations, varies from one to eleven days, with an average of 4.7 days.
5. None of the 17 men experimentally parasitized, nor the 3 nonparasitized controls, have developed dysentery.
6. From these results, the following conclusions appear warranted:
   a. *Entamœba coli*, unlike the *Amœbæ*, is a strict or obligatory parasite and cannot be cultivated on Musgrave and Clegg's medium.
   b. *Entamœba coli* is nonpathogenic, and consequently plays no rôle in the etiology of endemic tropical dysentery.

C. Twenty feeding experiments with *Entamœba histolytica* have been made on 20 volunteers, with the following results:
1. Cultures on Musgrave and Clegg's medium of the stools of men who have ingested *Entamœba histolytica* have been invariably negative.
2. Microscopic examinations, on the other hand, have shown *Entamœba histolytica*, after a short incubation period, in the stools of every man who became parasitized, and the entamœbæ have persisted in the stools of these men for an indefinite time.
3. Of the 20 men who ingested *Entamœba histolytica*, 17 became parasitized after the first feeding, 1 required 3 feedings before becoming permanently parasitized, and 2 who did not become parasitized at the first feeding were reserved as controls.
4. The incubation period of *Entamœba histolytica* in these experimentally parasitized men has been from one to forty-four days with an average of nine days.
5. In these experiments it has been possible to obtain:
   a. Encysted "*Entamœba tetragena*" exclusively in the stools of men who had ingested motile *Entamœba histolytica* only.

b. Motile *Entamœba histolytica* exclusively in the dysenteric stools of men who had ingested "*tetragena*" cysts only.

c. An alternation of "*tetragena*" cysts and motile *Entamœba histolytica* several times repeated in the stools of a man who had ingested "*tetragena*" cysts only and having attacks of dysentery alternating with normal stools.

6. Of the 18 men experimentally parasitized with *Entamœba histolytica*, 4, or 22.2 per cent, have up to the present time developed entamœbic dysentery.

7. The incubation period of the dysentery in these experimental infections has been twenty, ninety-five, eighty-seven, and fifty-seven days, respectively, with an average of 64.8 days.

8. No cases of dysentery have developed in men who ingested *Entamœba histolytica* from an acute case of entamœbic dysentery, from a liver abscess, nor in the 2 men who ingested *Entamœba histolytica* but who did not become parasitized with the entamœbæ.

9. All of the experimental dysenteries have been obtained after ingesting *Entamœba histolytica* from normal stools of "carriers." In 2 of the cases the infection was from "contact carriers" who had not, and have not subsequently, developed dysentery, and in one of the latter cases three hundred seventy-one days and the passage through 2 "contact carriers" intervened between the case of natural and the case of experimental entamœbic dysentery.

10. No cases of spontaneous entamœbic dysentery have occurred in this ward during the period of these experiments.

11. In consequence of the results obtained in these experimental infections of men with *Entamœba histolytica*, the following conclusions appear warranted:

a. *Entamœba histolytica*, like *Entamœba coli* and in contrast to the *Amœbæ*, is a strict or obligatory parasite and cannot be cultivated on Musgrave and Clegg's medium.

b. "*Entamœba tetragena*" Viereck is identical with *Entamœba histolytica* Schaudinn, and "*tetragena*" cysts are developed in the life cycle of *Entamœba histolytica*.

c. The large percentage of latent infections obtained in these experiments is wholly consistent with our clinical and pathological experience with entamœbic dysentery.

d. *Entamœba histolytica* is the essential etiologic factor in endemic tropical dysentery.

D. Information believed to be of the greatest value for the diagnosis, treatment, and prophylaxis of entamœbic dysentery has been obtained in this experimental investigation.

1. Since it has been determined that *Entamœba histolytica* is the specific etiologic agent, it will be possible to make an accurate laboratory diagnosis of entamœbic dysentery.

2. The distinction between the pathogenic *Entamœba histolytica* and the harmless *Entamœba coli* having been established, there will no longer exist an excuse for the indiscriminate treatment of all persons who show entamœbæ in their stools.

3. The relatively long incubation period of this disease and the ability to diagnose latent infections make it possible to anticipate with treatment an attack of entamœbic dysentery.

4. Since there is evidence that ipecac treatment, which is very efficient in relieving attacks of entamœbic dysentery and causing the entamœbæ to disappear temporarily from the stools, does not always kill all of the entamœbæ in the intestine, treatment should always be controlled by stool examinations for *Entamœba histolytica*. By this precaution, relapses, so common in entamœbic dysentery, can be forestalled.
5. The following data have been acquired upon which to base a rational prophylaxis of entamœbic dysentery:
   a. *Entamœba histolytica* is the essential etiologic agent in the disease.
   b. The specific entamœba is an obligatory parasite, and cannot propagate outside of the body of its host.
   c. The motile forms of this entamœba, which are passed in the bloody mucous stools in acute dysentery, quickly die and disintegrate and are probably, under natural conditions, incapable of withstanding passage through the human stomach.
   d. In consequence of the relatively long incubation period of entamœbic dysentery, the prevalence of chronic and latent infections, and the frequent failure of treatment to kill all of the entamœbæ in the intestine, "carriers" of *Entamœba histolytica* are common in endemic regions.
   e. These "carriers" are constantly passing in their stools large numbers of the resistant, encysted stage of *Entamœba histolytica*.
6. These facts make it probable that "carriers" of *Entamœba histolytica* constitute the chief, if not the sole, agents in the dissemination of entamœbic dysentery.
7. Prophylactic measures should, therefore, be directed toward "carriers" of *Entamœba histolytica*, and should include the following:
   a. The identification of "carriers" of *Entamœba histolytica* by the microscopic examination of the stools of convalescents, household servants, and other suspects or persons whose employment or associations make them particularly dangerous to the public health.
   b. The sanitary disposal of fæces.
   c. The treatment, controlled by microscopic examination of their stools, of all "carriers" of *Entamœba histolytica*.
8. Since the incubation period of entamœbic dysentery is usually long and latent infections are common, the most efficient personal prophylactic measure is frequent stool examinations, as an index for treatment, of all persons residing in endemic regions.

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ILLUSTRATIONS

(From water-color drawings by Teodosio S. Espinosa)

PLATE I

The figures in Plate I are all drawn from fixed and stained preparations at the magnification of Zeiss 1/12 oil-immersion objective, ocular 3, and tube length of 160 millimeters, and with the aid of a camera lucida.

FIG. 1. Motile form of a typical *Amœba*, cultivated from the Manila water supply. Note the small size, central arrangement of the chromatin in the nucleus, and the contractile vacuole.

2. Encysted form of the same species of *Amœba*. Note the small size and single nucleus with central arrangement of the chromatin.

3. Motile form of *Entamœba coli* from the stool of a healthy person. Note the dense, granular structure of the cytoplasm, the relatively large amount of chromatin and its peripheral arrangement in the nucleus.

4. Encysted form of *Entamœba coli*, from the stool of a healthy person. Note the large size, the relatively thick cyst wall, the 8 ring-form nuclei, and the absence of "chromidial bodies."

5. Motile form of *Entamœba histolytica*, from the stool of an acute case of entamœbic dysentery. Note the reticulated structure of the cytoplasm and the scanty chromatin in the ring-form nucleus.

6. The "*tetragena*" type of motile *Entamœba histolytica*, from a chronic case of entamœbic dysentery. Note the structure of the nucleus. It contains a heavier peripheral ring of chromatin—a part of which is detached from the nuclear membrane—than in the typical *histolytica;* and there is a central karyosome, consisting of a central granule surrounded by a circle of chromatin granules.

7. The preëncysted stage of *Entamœba histolytica*, from a "carrier" case. Note the small size, dense cytoplasm, and heavy peripheral ring of chromatin in the nucleus, which causes it to resemble a small *Entamœba coli.*

8. Encysted form of *Entamœba histolytica*, from a convalescent case of entamœbic dysentery. Note the small size, the cyst wall, the 4 ring-form nuclei, and the "chromidial body."

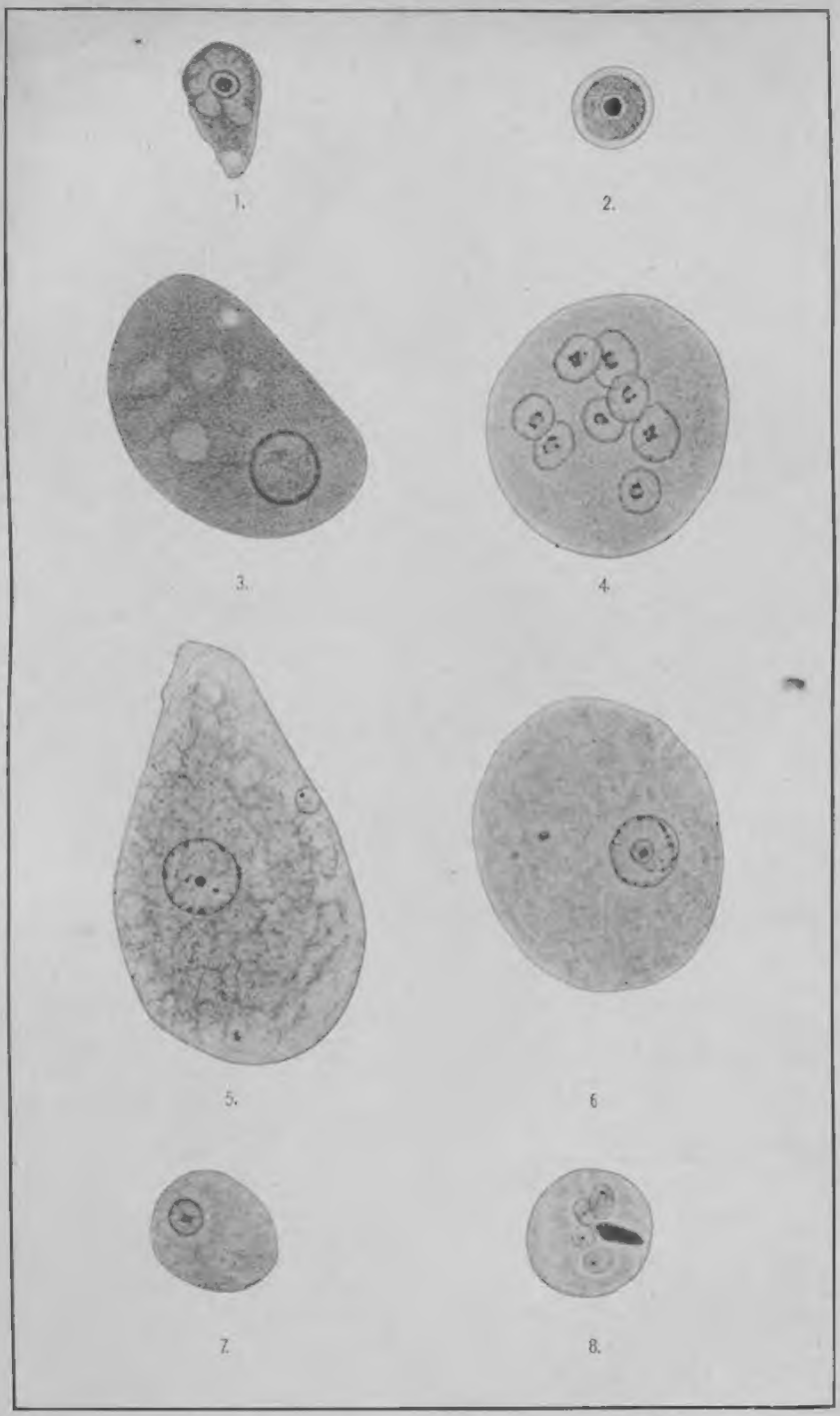

PLATE I. TYPICAL EXAMPLES OF AMŒBA AND ENTAMŒBA.

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